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(54) Title: PHARMACOLOGICALLY ACTIVE COMPOSITIONS OF CATECHOLIC BUTANES WITH ZINC

(57) Abstract

Pharmacologically active compositions of catecholic butanes and ionic zinc and the use thereof in the treatment of diseases and disorders of the skin, and their use as antibacterial and antifungal agents. The compositions are also useful in the treatment of benign, premalignant and malignant solid tumors, especially those of the skin.

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PHARMACOLOGICALLY ACTIVE COMPOSITIONS OF CATECHOLIC BUTANES WITH ZINC

Cross Reference to Related Applications

This application is a continuation-in-part of copending Application Serial No. 699,923 filed February 11, 1985, which is a continuation-in-part of Application Serial Number 578,501 filed April 9, 1984, now abandoned, which is a continuation-in-part of Application Serial Number 465,631 filed February 10, 1983, now abandoned, which is a continuation-in-part of Application Serial Number 365,781 filed April 5, 1982, now abandoned, which is a continuation-in-part of Application Serial Number 049,886 filed June 19, 1979, now abandoned, all the foregoing applications being fully incorporated herein by reference.

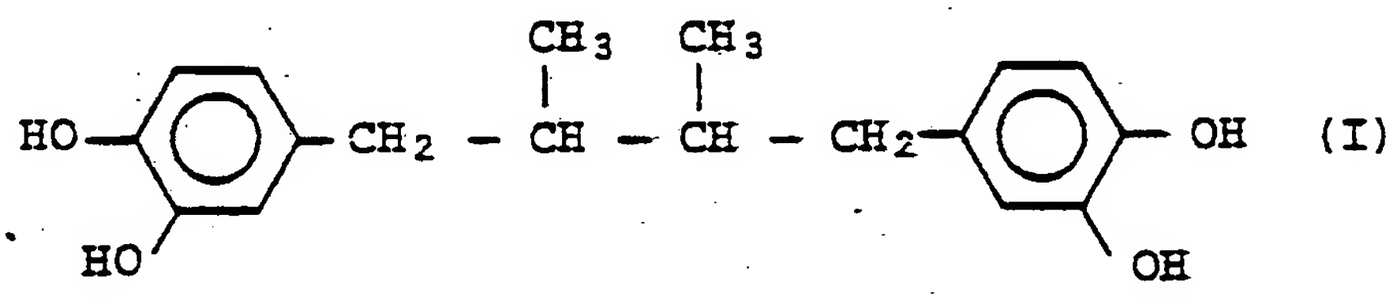
Field of the Invention

This invention relates to pharmacologically active compositions of catecholic butanes and ionic zinc and to the use thereof in the treatment of diseases and disorders of the skin, and to their use as antibacterial and antifungal agents. They are also useful in the treatment of benign, premalignant and malignant solid tumors, especially those of the skin.

Background

Mammals can be affected with a wide variety of skin disorders including bacterial and fungal infections and benign, premalignant and malignant growths. The systemic application of antibiotics has been commonly used as a treatment for bacterial infections in conditions such as acne and osteomyelitis. More recently, the topical use of certain antibiotics has been reported. For example, erythromycin in combination with zinc acetate has been reported as being useful in the topical treatment of

acne. Many other chemicals have been reported as having antimicrobial activity. Some of these, such as the meso form of nordihydroguaiaretic acid [meso-1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane] which occurs naturally, have been used as folk remedies. Unless otherwise indicated, NDGA is used herein to refer to the meso form of nordihydroguaiaretic acid. NDGA is a principal component in the creosote bush which has been used to make a tea used as a folk remedy for colds, rheumatism and other ailments for centuries. However, most of these chemicals including NDGA have not proven to be successful in the treatment of conditions such as acne and osteomyelitis. The general structure for nordihydroguaiaretic acid is given in Formula (I).



(I)

Methods of treating premalignant and malignant growths of the skin have often been traumatic. A common method of treating disorders such as actinic keratosis has been the application of liquid nitrogen to destroy the affected tissue. Epidermal tumors are commonly treated by physical removal through surgery. A method which has been used in the past is chemosurgery through the application of escharotic or fixative chemicals such as zinc chloride. This has not been found to be particularly effective because of the physical discomfort associated with the use of such materials. It also has the disadvantage of destroying both healthy tissue and the diseased tissue.

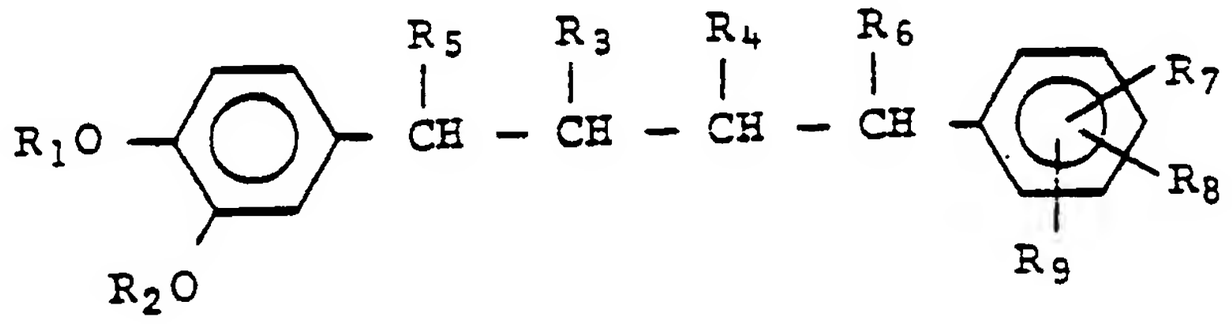
The use of known antitumor drugs has not been found to be particularly effective in the treatment of skin tumors since these drugs are commonly applied systemic-

ally resulting in substantial side effects due to their toxicity. NDGA was reported as providing a positive result against malignant melanoma. However, a clinical study was conducted by Smart, et al. in which human cancer patients ingested either a tea made from the creosote bush or doses of pure NDGA. This study indicated that neither NDGA nor the tea were effective anticancer agents and in some cases caused a stimulation of tumor cell growth. C. R. Smart, et al. Rocky Mountain Medical Journal, Nov. 1970, pp. 39-43. This confirmed earlier screening studies of NDGA conducted by the Cancer Chemotherapy National Service Center which obtained negative results when NDGA was tested against several types of cancer cells.

Surprisingly, we have found that nordihydroguaiaretic acid in a pharmaceutical composition containing ionic zinc is effective in treating disorders of the skin including bacterial infections which occur in acne and in osteomyelitis when applied to the situs of the disorder. Such compositions are also effective in treating benign, premalignant and malignant growths of the skin without the detrimental side effects associated with chemosurgical techniques, when applied topically to or injected into the growth.

Summary of the Invention

In a compositional aspect, this invention relates to pharmaceutical compositions comprising a catecholic butane of formula



(II)

wherein R_1 and R_2 are independently H, C_1-C_6 alkyl, or a C_7 or lower acyl;

R_3 and R_4 are independently H, CH_3, or C_2H_5;
R_5 and R_6 are independently H or OH; and
R_7, R_8 and R_9 may be attached at any separate position C_2-C_6 of the benzene ring and are independently H, OH, or OR_1 (wherein R_1 is as above);
and a pharmaceutically acceptable source of ionic zinc.

In another composition aspect, this invention relates to pharmaceutical compositions adapted for topical administration comprising, in admixture with a pharmaceutically acceptable carrier, a mixture of (i) a catecholic butane of Formula (II) and (ii) a pharmaceutically acceptable source of ionic zinc.

In preferred composition aspect, this invention relates to such compositions, adapted for topical application to a situs or injection into the interior of the situs, comprising nordihydroguaiaretic acid and a source of ionic zinc, and to such compositions in combination with a pharmaceutically acceptable carrier.

In a method of use aspect, this invention relates to a method for inhibiting the proliferation of abnormal cells in a mammal which comprises applying directly to the situs of the abnormal cells an amount of a composition of this invention effective to inhibit said proliferation.

In another method of use aspect, this invention relates to a method of promoting the healing of a lesion in the tissue of a mammal which comprises applying thereto an amount of a composition according to this invention effective to promote the healing thereof.

In a further method of use aspect, this invention relates to a method of increasing the oxidative stability of a catecholic butane of Formula (II) which comprises mixing with said catecholic butane an oxidation inhibiting amount of ionic zinc.

In a still further method of use aspect, this invention relates to a method of enhancing the retention

time of a catecholic butane at the situs of an affliction to which said catecholic butane is applied, which comprises applying said catecholic butane as a composition according to this invention containing an amount of said source of ionic zinc effective to enhance said retention time.

Detailed Description of the Invention

The term "source of ionic zinc" as used herein means a compound comprising ionic zinc in salt or chelated form, as opposed to metallic zinc. That source can be or can include the catecholic butane itself. When used herein, "zinc" means zinc in its ionic or divalent state and not metallic zinc.

The terms "afflicted situs" or "situs" as used herein refer to a localized area of pathology, infection, lesion or wound, or abnormal cells including solid tumors, and the immediately surrounding area.

The term "applying" as used herein embraces both topical applications to a surface of the situs and injection into the interior of the situs.

The term "mammal" as used herein includes feline, canine, equine, bovine, rodent and primate species, including cats, dogs, horses, rats, mice, monkeys and humans. Other animals, e.g., birds, can also be successfully treated with the compositions of this invention.

The term "abnormal cells" embraces both benign, premalignant and malignant cells. Examples of the former include the cells associated with adenomas, papillomas, etc. Examples of premalignant cells include actinic keratosis.

The term "proliferation" refers to the reproduction or multiplication and growth of cells.

The term "escharotic" means a corrosive or caustic agent which is capable of killing healthy, living cells.

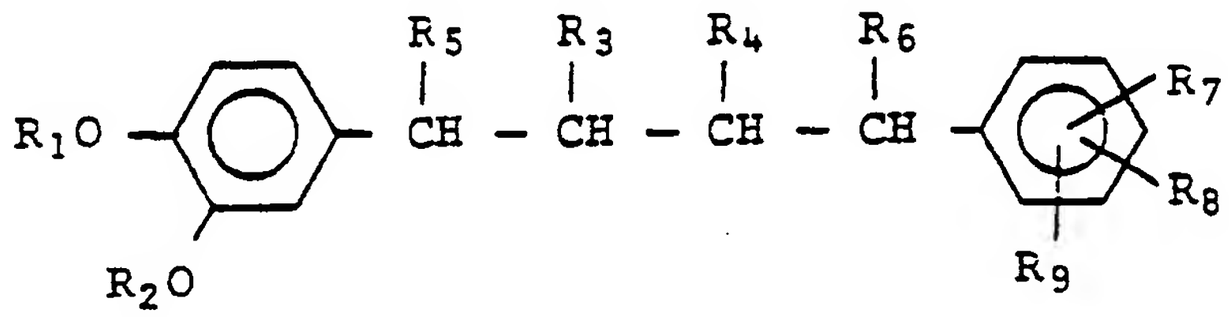
The term "nonescharotic concentration" means a concentration of the source of ionic zinc which does not kill living cells upon contact, e.g., as does zinc chloride when employed as an escharotic agent, e.g., at a concentration of about 40 weight percent or higher, depending on the delivery vehicle.

Compositions comprising a catecholic butane and zinc are particularly effective for the treatment of a variety of skin disorders and solid tumors. Improved results are obtained when the affected area is directly contacted with the instant compositions. With such compositions it has also been found that, surprisingly, the catecholic butane is retained by the tissue at the treatment site for a significant period of time before being distributed throughout the organism. This unexpected property of the instant compositions can increase the effectiveness of the treatment and also minimize any detrimental side effects of the components. Additionally, the combination of a catecholic butane and zinc allows a reduction in the concentration of each individual component while maintaining the efficacy of the composition. This reduction in the dosage level of the individual components obtained by combining the two active ingredients increases the safety of the composition. The instant compositions have been found to unexpectedly provide improved restoration of integrity to injured tissue. The presence of zinc has also been found to substantially increase the stability of the catecholic butane to oxidative reactions. The catecholic butane and zinc also unpredictably show no evidence of pharmacological antagonism.

The novel compositions of this invention are useful as antimicrobial, antifungal, antiviral and antitumor agents, as lesion healing promoting agents, e.g. for skin ulcers such as decubitus ulcers and lesions associated with osteomyelitis. They are useful in the treatment of keratoses, especially actinic keratosis including senile

keratotic lesions. They are useful in treating a wide variety of premalignant and malignant skin tumors, basal cell carcinoma, squamous cell carcinoma and a diversified variety of melanotic lesions which are premalignant or malignant as well as certain cutaneous tumor manifestations of otherwise systemic diseases. The compositions have been found to be effective against solid tumors arising from all three embryonic tissue types, namely squamous cell carcinoma, e.g. lung carcinoma, arising from the ectodermal layer; adenocarcinomas, e.g. breast, renal and colon cancer, arising from the endodermal layer; and melanoma and brain cancers, arising from the mesodermal layer.

The catecholic butanes useful in the compositions of the instant invention are of the Formula (II)



(II)

wherein R_1 and R_2 are independently H, C_1-C_6 alkyl, or C_7 or lower acyl;

R_3 and R_4 are independently H, CH_3, or C_2H_5;

R_5 and R_6 are independently H or OH; and

R_7, R_8 and R_9 may be attached at any separate position C_2-C_6 of the benzene ring and are independently H, OH, or OR_1 (wherein R_1 is as above).

Illustrative classes of compounds within the scope of Formula (II) are those wherein:

a) one or more of R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are H, e.g., those wherein R_5 is H, R_5 and R_6 are H or R_5, R_6 and R_7 are H and R_8 and R_9 are OH or OR_1;

b) R_3 and R_4 each are CH_3 or C_2H_5 including those of a), especially those wherein R_5, R_6 and R_7 are H and/or R_8 and R_9 are OH or OR_1;

c) R_1 and R_2 are acyl, e.g., hydrocarbonacyl, preferably, alkanoyl, e.g., acetyl, propionyl, etc., including those of a) and b);

d) R_1 and R_2 are alike and R_8 and R_9 are OR_1, including those of a), b) and c); and

e) The compound is in the form of a single optical isomer, a mixture of such isomers, e.g., a racemic mixture, or a mixture of diastereoisomers, including each of a), b), c) and d).

As used herein, alkyl represents, inter alia, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, n-hexyl, etc.

Acyl represents groups having the general formula RCO-, e.g., acetyl (CH_3CO-), propionyl (CH_3CH_2CO-), butyryl ($CH_3CH_2CH_2CO$-), 3,3-dimethylbutyryl [$(CH_3)_3CCH_2CO$-], etc. When the catecholic butane compound is named as a substituted phenyl, the corresponding groups are acetoxy (CH_3CO_2-), propionyloxy ($CH_3CH_2CO_2$-), and butyryloxy ($CH_3CH_2CH_2CO_2$-), 3,3-dimethylbutyryloxy [$(CH_3)_3CCH_2CO_2$-], etc.

Examples of catecholic butanes include the d-, l-, racemic mixture of d- and l-, and meso-isomers of 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dihydroxyphenyl)butane; 1,4-bis(3,4-dimethoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-diethoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropoxyphenyl)-2,3-dimethylbutane; 1-(3,4-dihydroxyphenyl)-4-(3',4',5'-trihydroxyphenyl) butane; 1,4-bis(3,4-diacetoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dibutyroyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-divaleroyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipivaloyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,3-dimethylbutyryloxylphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dihydroxyphenyl)-2-methylbutane; 1,4-bis(3,4-dihydroxyphenyl)-2-methyl-3-ethylbutane; and 1-(3,4-dihydroxyphenyl)-4-phenylbutane. It is contem-

plated that mixtures of these catecholic butanes can be used in the instant compositions.

The zinc is present in the instant compositions as a cation, e.g., as a salt or a chelate of the catecholic butane itself or as pharmaceutically acceptable salt of another toxicologically acceptable anion, or as a mixture thereof. Pharmaceutically acceptable salts include those of inorganic acids, e.g., nitrate, sulfate, acetate, halides and phosphates, and those of organic acids, e.g., acetate, benzoate, citrate, caprylate, gluconate, etc., and mixtures thereof. Zinc chloride is especially preferred. As stated above, the term "zinc" as used herein means ionic zinc, rather than zinc metal.

The molar ratio of catecholic butane to zinc in the compositions of this invention can vary over a wide range. Ordinarily the molar ratio is between 100:1 and 1:100, more commonly between about 10:1 and 1:20 and even more commonly between about 5:1 and 1:15, and most commonly between about 3:1 and 1:10. The molar ratio preferably is such that at least one of the catecholic butane and zinc is present in the composition at a concentration effective to inhibit the proliferation of abnormal cells and the other of the two is present therein at a concentration effective to enhance that proliferation inhibiting activity, when the composition is applied in effective amounts to the situs of those cells. However, the molar ratio can be any that results in the composition exhibiting one or more of antifungal, antibacterial, antiviral and anti-tumor activity. In one aspect of this invention, the zinc is present at a concentration which prolongs the half-life of the catecholic butane at the situs of application. In another aspect, the zinc is present at a concentration which inhibits oxidation of the catecholic butane. In a further aspect, the catecholic butane and zinc are present in concentrations to promote healing of a wound

or lesion contacted therewith. The preferred molar ratio range depends upon the particular condition being treated as well as the method of delivery of the composition to the treatment site. The preferred range can be determined by normal pharmacological screening methods used in the art such as against the particular bacteria strain or strain of tumor cells. If desired, an excess of the zinc or the catecholic butane can be used as appropriate for the specific condition being treated.

The instant compositions can be applied topically to or injected into the treatment site, e.g., solid tumor, lesion or wound. When used for topical applications, the catecholic butane and the source of ionic zinc are usually formulated with a pharmaceutically-acceptable carrier. As used herein the term "pharmaceutically-acceptable carrier" refers to a material that is non-toxic, generally inert and does not adversely affect the functionality of the active ingredients. Carrier materials are well known in the pharmaceutical formulation art and include those materials referred to as diluents or vehicles. The carrier can be an inorganic or organic material and should have sufficient viscosity to allow spreading of the composition and provide good adherence to the membrane to which it is topically applied. Examples of such carriers include without limitation polyols such as glycerol, propylene glycol, polyethylene glycol, preferably of a molecular weight between about 400 and about 8000, suitable mixtures thereof, vegetable oils, etc. The viscosity of the formulation can be adjusted by methods well known in the art, for example by the use of a higher molecular weight polyethylene glycol.

In addition to the catecholic butane, source of ionic zinc and carrier, the formulation can contain pharmacologically-acceptable additives or adjuvants such as antimicrobial agents, e.g. methyl, ethyl, propyl, and butyl esters of para-hydroxybenzoic acid, as well as

chlorobutanol, phenol, ascorbic acid, etc. The formulation can also contain thickening or gelling agents, emulsifiers, wetting agents, coloring agents, buffers, stabilizers and preservatives including antioxidants such as butylhydroxyanisole. The formulation can also contain penetration enhancers such as dimethyl sulfoxide, long-chain alcohols such as nonoxynol, long-chain carboxylic acids, propylene glycol, N-(2-hydroxyethyl)pyrrolidone, 1-dodecyl-azacycloheptan-2-one, and the like. Depending on the method of application and the disease being treated, it may be desirable to use absorption-delaying agents such as aluminum monostearate and gelatin.

The composition of the formulation can be adjusted using components well-known in the formulation art to provide a pharmaceutical formulation which is a gel, cream, ointment, solid, liquid, semi-solid, etc. The particular physical form of the formulation depends on the desired method of treatment and the patient to be treated.

For administration by injection, the composition is formulated as a solution or suspension having a low enough viscosity to be injected. The composition suitable for injectable use must be sterile and fluid to the extent that easy syringe injection exists. It should also be stable under conditions of manufacture and storage and be preserved against contamination by microorganisms. Additionally, the pH of the composition must be within a range which does not result in tissue damage.

The concentrations of the catecholic butane and the ionic zinc in a particular formulation depend on the condition being treated, the method of application, i.e. topical or injection, the rate of delivery of the active ingredient(s) to the treatment site, and the number of applications of the formulation which can be used. Additionally, certain catecholic butane compounds are more effective in treating particular conditions than are

other analogs. The optimum amount of a specific catecholic butane for treating a condition cannot be predicted at this time. However, an effective range can readily be determined by procedures known to those skilled in the art and explained elsewhere herein. It has been found that ordinarily a lower concentration of catecholic butane and ionic zinc can be used when treating a microbial infection than when treating a solid tumor. The concentration of ionic zinc in the formulation can likewise depend upon the condition being treated and the particular catecholic butane or combination of butanes being used. As discussed hereinabove, it may be desirable to have a substantial excess of one component, for example ionic zinc, present in the formulation in order to effectively treat the particular condition.

In practice, it is preferred that a formulation contain the lowest concentrations of catecholic butane and ionic zinc which effectively treat the condition with the desired number of applications, i.e. a lower effective dose rate can be tolerated if multiple applications are used. This low concentration limit is dependent upon the delivery effectiveness of the carrier vehicle. Preferably, the catecholic butane and zinc together comprise between about 0.5 and about 80 weight percent of the formulation. Recognizing that it may be possible to use lower concentrations depending on the delivery of the carrier, it is expected that a formulation for treating microorganisms or fungi would ordinarily contain between about 0.001 and about 20 weight percent of catecholic butane and between about 0.001 and about 30 weight percent zinc. In the treatment of solid tumors, it is ordinarily expected that the formulation contain between about 0.1 and about 30 weight percent catecholic butane and between about 0.05 and about 35 weight percent zinc. Preferably at least one of the catecholic butane and the zinc is present in the formulation at a concentration of

at least about 0.5 weight percent, more preferably at least about 1.0 weight percent. As used herein, the weight percent in the formulations refer to the concentrations of materials being effectively delivered to the treatment site. As stated above, it is contemplated that formulations can be prepared that have significantly higher concentrations of catecholic butanes and zinc depending upon the carrier and additives being used. If the carrier substantially retains the catecholic butane and zinc or releases them at a slow rate, the concentrations of these materials in the formulation can be substantially increased and in fact may have to be substantially increased in order to provide an effective treatment. The concentrations of active ingredients in a particular formulation required to provide a particular effective dose (ED) can be generally determined by a person skilled in the pharmaceutical formulation art based upon the properties of a carrier and the particular additives introduced into the formulation. It is also expected that a formulation which is being applied topically can contain a higher concentration of catecholic butane and zinc than a composition being injected, for example into a solid tumor.

A preferred embodiment of the instant invention comprises compositions containing nordihydroguaiaretic acid, i.e. 1,4-bis(3,4-dihydroxylphenyl)-2,3-dimethylbutane, and zinc chloride. This combination has been found to be particularly effective in treating acne and Propionibacterium acnes, decubitus ulcers, osteomyelitis, actinic keratosis and solid tumors. Since zinc chloride at high concentrations is an escharotic material, it is preferred that the concentration of zinc chloride delivered to the treatment site be maintained below a concentration which is escharotic to the healthy tissue. Although the effective concentration of zinc chloride as well as nordihydroguaiaretic acid delivered to the treat-

ment site depends upon the carrier and other additives included in the formulation, ordinarily the concentration of nordihydroguaiaretic acid in the formulation will range from about 0.01 to about 40 weight percent and the concentration of zinc chloride in the formulation will range from about 0.01 to about 35 weight percent. These ranges are provided by way of description and not by way of limitation since it is recognized that the concentration can be adjusted over a wide range depending on the carrier material, number of applications used, etc., as described hereinabove.

The instant compositions have the advantage of the beneficial and unexpected interaction between the catecholic butane and ionic zinc. This beneficial relationship is not understood at this time; but it allows the concentrations of the catecholic butane and zinc to be reduced to lower, more toxicologically acceptable levels while obtaining comparable or superior results to the use of higher concentrations of individual components. Thus, the concentration of zinc chloride can be reduced to below an escharotic level in the formulation.

The pH of the formulation can be important in assuring stability of the catecholic butane as well as assuring that the formulation is physiologically acceptable to the patient. Many of the catechols, particularly nordihydroguaiaretic acid, are susceptible to oxidation, for example by air. Such oxidation can result in discoloration of the formulation rendering it unacceptable for pharmaceutical use. These catechols are more stable against oxidation at lower pH levels. Therefore, it is preferred that if the formulation is to be exposed to oxidizing conditions the pH be maintained below about 7 and preferably below about 6 in order to provide maximum stability for the catechol against oxidation. However, if oxidizing conditions can be avoided, for example by storage of the formulation under an inert atmosphere such

as nitrogen, a higher pH can be used. The pH of the formulation can be maintained through the use of toxicologically-acceptable buffers. Such buffers are well known in the pharmaceutically formulation art.

It has been found that the presence of ionic zinc in a catecholic butane formulation can substantially retard the rate of oxidation of the catechol, i.e. increase the stability of the catecholic butane to oxidation. This has significant advantages in that the introduction of unknown oxidation products of the catecholic butanes is minimized and the shelf-life of the catecholic butane compositions is increased. While not intending to be bound by a possible explanation of this not fully understood phenomena, experimental evidence set forth in the instant examples indicates that the ionic zinc serves to stabilize the semiquinone free radical and radical-anion intermediates formed during the oxidation process possibly by forming a complex with the catechol. Surprisingly, zinc ions dramatically decreased the decay rates of these radicals compared to other metal ions tested. Consequently, the stability to oxidation of a catecholic butane formulation such as one containing nordihydroguaiaretic acid can be increased by the addition of zinc ions in the form as discussed hereinabove. It is expected that the presence of ionic zinc in a molar ratio zinc to catecholic butane of about 1:50 can increase the stability of the catechol; however, it is preferred that the molar ratio zinc to catechol be at least about 1:5, and most preferably at least about 1:2 with an excess of zinc contemplated as being most beneficial.

The compositions of the instant invention have also been found to be useful in the treatment of lesions, draining lesions, and draining wounds which show impaired healing. As used herein the term "lesion" refers to any pathological or traumatic discontinuity of tissue. A "wound" is a lesion which results from a bodily injury

caused by physical means. Lesions which do not readily heal can be manifestations of conditions, diseases or infections, for example, cutaneous ulcers, osteomyletis, acne vulgaris, draining fistulas, etc. Not uncommonly, lesions do not heal properly and continue to drain which results in discomfort to the patient and a continued threat of severe infection. Such conditions in which tissue does not readily grow to heal the lesion or wound can be the result of bacterial infection or other causes not fully understood. Exposed areas, created by the sloughing off of necrotic matter, generally result in pus formation (suppuration). Although the exact mechanism is unknown, direct contact of the exposed area of the lesion with the instant compositions has been found in clinical studies to substantially aid the healing process, possibly by inducing the formation of granulation tissue. The instant compositions are beneficial in promoting healing of lesions in patients having serum zinc levels within the range generally accepted as being normal. This promotion of healing has significant advantages, for example, in the treatment of solid tumors directly or the situs from which such tumors have been surgically removed in that healing is promoted concurrently with inhibiting the proliferation of any tumor cells which might remain at the site of surgery.

In topical applications the instant compositions are applied to the affected area or afflicted situs of the patient. The term "topical" refers herein to the surface of the epidermal tissue, especially the skin, the surface of tumors on the skin which have been debrided or otherwise modified, as well as sites from which solid tumors have been removed either from the skin or internally. The instant compositions can be particularly useful in conjunction with surgery for removal of internal cancers to eradicate residual tumor cells and act as a prophylactic against local recurrence and metastatic

spread of the tumor. The instant compositions can be used instead of surgery when there are cosmetic considerations due to the normally improved appearance of healed situs treated with the instant compositions compared to surgery alone.

Application by injection can be used for treatment of solid tumors in which removal by surgery is not desired or for which surgery is not medically advisable. In this procedure the instant composition is injected directly into the tumorous growth. The injection may be accomplished at a number of sites in the growth in order to provide the maximum contact between the instant composition and the tumorous cells.

As used herein the term "solid tumor" refers to tumors in which a plurality of tumor cells are associated with one another, i.e. contiguous and localized within a confined site. This is to be contrasted with "fluid" or "hematogenous" tumors in which the tumor cells occur primarily as unassociated or individual cells, e.g. leukemia. Solid tumors generally propagate on host tissues such as the epithelial, the connective and supportive tissues as well as other tissues located throughout the body. Examples of epithelial tumors include papillomas and carcinomas such as squamous cell carcinoma, basal cell carcinoma, adenoma, adenocarcinoma, cystadenoma and cystadenocarcinoma. Examples of supportive and connective tissue tumors include sarcomas and their benign counterparts such as fibrosarcoma, fibroma, liposarcoma, lipoma, chondrosarcoma, chondroma, leiomyosarcoma and leiomyoma. Examples of other tissue tumors include gliomas (brain tumors) and malignant melanomas.

The compositions of the instant invention have been found to be particularly effective against the following solid mammalian tumors: human tumors including malignant melanoma, squamous cell carcinoma, lung squamous cell carcinoma, breast adenocarcinoma, glioma, glioastrocy-

toma, renal-cell carcinoma, colon, and basal cell epithelioma; canine tumors including mast cell carcinoma, squamous cell carcinoma, mammary adenoma, breast adenocarcinoma, perianal adenocarcinoma, perianal adenoma, sebaceous adenoma, and basal cell carcinoma; and equine tumors including papilloma, malignant melanoma, sarcoid and squamous cell carcinoma.

In order to determine the efficacy of a composition as an antimicrobial, antiviral, antifungal or antitumor agent, the composition is commonly initially tested by in vitro screening methods. When tested against microorganisms, the composition is commonly applied to a colony at different concentrations and the kill ratio determined. In the treatment of tumors, initial screening is commonly done by the human tumor clonogenic assay. It has been reported that clinical correlations from retrospective analysis and prospective clinical trials with such clonogenic assays have indicated that there is a 60 to 70 percent correlation between in vitro sensitivity and clinical response. The studies have also indicated that there is a greater than 90 percent correspondence between in vitro resistance and treatment failure. However, the screening of new antitumor agents is still primarily being conducted using a variety of tumor models in vivo. The National Cancer Institute is currently using in vivo tumor models which include the L-1210 lymphocytic leukemia, B-16 melanoma, M-5076 carcinoma, 3 transplantable murine tumors, and the MX-1 human mammary tumor xenograph.

In preparing a formulation suitable for topical application, the catecholic butane is normally mixed with a suitable solvent. Examples of solvents which are effective for this purpose include ethanol, acetone, acetic acid, aqueous alkaline solutions, dimethyl sulfoxide, glycerine, glycerol, propylene glycol, suitably high boiling ethers, nonoxynol, polyethylene glycol, etc.

The zinc ions, commonly in the form of a toxicologically-acceptable salt, are mixed with a suitable solvent such as water or polyethylene glycol of low molecular weight, e.g. 200-400. The ionic zinc can be added in the form of readily available salts such as acetates or other aliphatic acid salts while the preferred anion, e.g. chloride, can be added in the form of its readily available salts such as sodium chloride. In the event there is not complete solubilization, the mixture can be milled to obtain a fine suspension.

The catecholic butane composition and the source of ionic zinc are mixed in appropriate amounts to achieve the desired concentrations. Additives, adjuvants, other carriers, etc., can be introduced at any stage of the preparation as appropriate. When the formation of a metal chelate or complex is desirable, the ordering of mixing of ingredients and the pH of the formulation can be critical. When chelates or complexes are desired, compounds which can serve as counter-ligands are preferably provided so that discreet "molecular" entities are formed rather than polymers of indeterminant length. Such counter-ligands include ethylenediamine tetraacetic acid (EDTA), ethylenediamine diacetic acid (EDDA), ethylenediamine, ammonia, ethanolamine, amino acids, etc.

The following examples are included by way of illustration and not by way of limitation.

EXAMPLES

The following examples are illustrative of the preparation and use of the compositions of this invention in the treatment of various types of tumors, and as inhibitors of various infectious agents.

In Examples 1-14, wherein reference is made to the testing of mixtures for antitumor activity against B-16 melanoma and Sarcoma-180 solid tumor growth in mice, the following procedures were utilized. To the extent that a

particular example modified the procedure, such modification will be indicated in the particular example.

Both types of tumors were grown intradermally or subcutaneously in the mice. The B-16 melanoma was grown in BDF_1 mice and the S-180 tumor was grown in ICR mice. Each mouse was injected intradermally with about 0.01 ml of a saline suspension containing about 1×10^6 cells of the tumor cells per 0.01 ml into a preshaven area on the back of the neck of the mouse. The tumors were allowed to grow until they had an approximate size of about 25-100 mg, calculated by the length of the tumor multiplied by the width and height of the tumor measured in millimeters and dividing the product by two. On the first day of treatment, the animals with tumor sizes outside of the size range were culled and the remaining animals were randomly divided into control and test groups. When the tumors had reached the appropriate size, usually at about day six, the tumors were punctured uniformly and then treated with either a test compound or a control by topical application to the surface of the tumor. Generally, two topical applications were made 24 hours apart. The materials were applied to obtain from about a 1 to about 2 mm coating over the surface of the tumor. The animals were thereafter observed and their weights and the size of their tumors were periodically measured.

The results of each of the experiments include the following:

(a) the starting number (n) of animals within a treatment group of an experiment;

(b) the average tumor size in milligrams of the animals treated with the mixture and the average tumor size of the control animals;

(c) the ratio multiplied by 100 of the average size of the tumors of the treated animals to that of the control animals (T/C), wherein T = average size of

treated mice and C = average tumor size of control mice;

(d) the percentage of both treated and control animals clear of tumor; and

(e) the percentage of animals of the original number surviving.

The later three measurements for a particular experiment were all taken at the same time and range generally from 21 to about 33 days after tumor inoculation. A T/C value of 42 or less is indicative of activity. In all of the following tables for Examples 1-14, the control results are given in parenthesis ( ).

Unless otherwise indicated, the nordihydroguaiaretic acid used in the instant Examples was the meso-isomer and is designated NDGA. Other isomers are indicated, e.g., d,l-NDGA.

EXAMPLE 1

A variety of mixtures containing nordihydroguaiaretic acid (NDGA), quercetin, zinc chloride, ascorbic acid or sodium ascorbate were prepared and formulated into a polyethylene glycol (PEGO) base to obtain an appropriate consistency for application. The mixtures were prepared by dissolving the NDGA in ethanol while warming and stirring. After the NDGA was dissolved the quercetin was added with continued stirring and warming. Upon its dissolution a small amount of water was added and then zinc chloride added or, alternatively, zinc chloride dissolved in water was slowly added to the mixture with continued stirring and warming. Finally, the ascorbic acid or sodium ascorbate previously dissolved in water was then slowly added with continued stirring. Thereafter, the entire mixture was continued to be heated until sufficient solvent had been evaporated to obtain a mixture weighing between about 130 and 140 wt % of the weight of the dry ingredients. The composition in wt/wt

percentages of the mixtures thus prepared are given in Table 1. Due to the fact that the weight of the final mixture varied between 130 and 140 wt % of the dry ingredients, the weight percentages are approximate.

TABLE 1

Mixture	$ZnCl_2$	meso-NDGA	Quercetin	Na Ascorbate	Solvent	PEGO
1	47.4	11.8	11.8	7.9	-	21
2	39.6	19.8	0	6.7	12.5	20.8
3	41.4	20.7	0	6.9	21.3	10.6
4	36.4	9	9	5.4	18.2	21.8
5	46	11.5	11.5	7.7	3.0	20.4
6	37	9.2	9.2	6.0	2.4	21.1
7	33.3	8.3	8.3	5.6	23.3	21.1
8	29.9	7.5	7.5	5.1	-	50
9	37	9.2	9.2	6.0	2.4	21.1
10	40.3	10.1	0	6.7	16.8	26.2
11	40.3	10.1[1]	0	6.7	16.8	26.2
12	40	10	0	1	14.6	33.7
13	40	6.7	0	6.7	15.6	31.1
14	40	3.3	0	6.7	15.6	34.4
15	31	7.6	0	5.2	12.9	43.0
16	18.8	4.6	0	3.1	7.9	65.4
17	35.5	8.9	0	5.9	17.8	32.0
18	40	10	0	3.3	15.6	31.1
19	27.6	10.2	0	6.9	12.6	42.5
20	14.3	10.6	0	7.1	9.5	58.3

[1] d,l-NDGA used.

EXAMPLE 2

The mixtures of Example 1 were tested for antitumor activity against B-16 melanoma and S-180 solid tumor grown in vivo in mice according to the previously described procedure. The results of the tests against B-16 melanoma are given in Table 2 and the T/C tumor size, etc. were determined between day 21 and day 24 post tumor inoculation.

The results of the testing against S-180 tumor are given in Table 2.

TABLE 2

B-16 Melanoma

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
1	6[1]	6.7	46±104 (683±425)	66 (0)	66 (25)
1	10	2.7	6±18 (207±169)	90 (0)	100 (70)
2	6	12	579±1047 (4752)[2]	50 (0)	83 (33)
4	3	4	47±82 (1085±247)	66 (0)	
5	8[1]	15	106±260 (683±425)	75 (0)	50 (25)
5	3	0	0	100 (0)	67 (33)
5	3	74	479±29 (647±421)	0 (0)	100 (67)
6	3	106	684±332 (674±421)	33 (0)	100 (67)
10	8	0	0 (982±530)	100 (0)	100 (100)
10	9	11	0 (799±677)	80 (0)	55 (60)
10	10	0	0 (2876±1202)	100 (0)	100 (100)
10	10	7	200±497 (2876±1202)	70 (0)	100 (100)
11	5	0	0 (799±677)	100 (0)	60 (60)
11	8	0	0 (982±530)	100 (0)	62 (100)

[1]Tumor was treated topically once daily for 5 days.

[2]Two of three controls died.

TABLE 2 (Cont)

S-180 TUMOR

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
5	10	25[4]	308±410 (1239±551)	0 (0)	100 (100)
5	10	46[2]	575±421 (1252±1184)	20 (0)	90 (100)
7	5	95[3]	716±480 (752±511)	0 (0)	100 (100)
8	10	45[3]	555±376 (1167±436)	10 (0)	90 (100)
9	10	104[4]	1285±713 (1239±551)	10 (0)	100 (100)
10	9	69[5]	655±605 (952±501)	11 (0)	100 (100)
10	10	55[5]	526±408 (952±501)	20 (0)	100 (100)
10	10	46[4]	656±660 (1435±512)	70 (0)	100 (80)
10	10	15[4]	221±308 (1435±512)	50 (0)	100 (80)
10	10	26[4]	373±616 (1435±512)	60 (0)	100 (80)
10	10	11[4]	159±241 (1435±512)	60 (0)	100 (80)
10	10	3[4]	51±108 (1435±512)	70 (0)	100 (80)
10	10	22[7]	222±354 (997±421)	44 (0)	100 (10)
10	10	28[7]	285±421 (997±421)	50 (0)	100 (10)
10	10	5[5]	44±108 (952±501)	80 (0)	100 (100)
10	10	51[5]	488±501 (952±501)	30	100

TABLE 2 (Cont)

S-180 TUMOR

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
10	10	83^{7}	803±423 (966±535)	0 (10)	100 (100)
10	10	56^{5}	814±803 (1450±594)	20 (0)	100 (90)
10	10	29^{7}	240±407 (1417±685)	70 (0)	100 (100)
10	13	13^{4}	124±256 (969±655)	70 (0)	100 (100)
10	10	18^{5}	171±287 (952±501)	60 (0)	100 (100)
12	10	51^{7}	498±516 (966±535)	10 (10)	100 (100)
12	10	46^{5}	674±479 (1450±594)	10 (0)	100 (90)
13	10	40^{7}	386±359 (966±535)	30 (10)	100 (100)
13	10	29^{5}	426±400 (1450±594)	30 (0)	100 (90)
14	10	27^{7}	259±214 (966±535)	30 (10)	100 (100)
14	10	42^{5}	612±535 (1450±594)	20 (0)	100 (90)
15	10	25^{7}	354±510 (1417±685)	55 (0)	100 (100)
15	10	13^{4}	124±256 (969±655)	70 (0)	100 (100)
16	9	53^{7}	750±414 (1417±685)	0 (0)	100 (100)
16	10	92^{4}	890±787 (969±655)	30 (0)	100 (100)

TABLE 2 (Cont)

S-180 TUMOR

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
17	10	15[6]	82±120 (550±184)	60 (0)	100 (100)
17	10	65[7]	710±468 (1091±547)	0 (0)	88 (80)
17	5	12[8]	80±98 (654±335)	55 (0)	100 (77)
17	5	26[3]	195±249 (752±511)	40 (0)	100 (100)
17	10	13[5]	127±292 (952±501)	80 (0)	100 (100)
18	10	19[7]	189±252 (966±535)	40 (10)	100 (100)
18	10	16[5]	232±288 (1450±594)	30 (0)	100 (90)
19	10	93[7]	902±353 (966±535)	0 (10)	80 (100)
19	10	49[5]	714±272 (1450±594)	0 (0)	100 (90)
20	10	114[7]	1101±488 (966±535)	0 (10)	100 (100)
20	10	129[5]	1165±485 (1450±594)	0 (0)	100 (90)

[1]Tumor was treated topically once daily for 5 days.
[2]Results determined 4 weeks post tumor inoculation.
[3]Results determined 3 weeks post tumor inoculation.
[4]Results determined 23 days post tumor inoculation.
[5]Results determined 30 days post tumor inoculation.
[6]Results determined 16 days post tumor inoculation.
[7]Results determined 22 days post tumor inoculation.
[8]Results determined 31 days post tumor inoculation.

EXAMPLE 3

Several different mixtures of NDGA and zinc chloride were prepared. Mixtures 21-24 were prepared by dissolving the NDGA in warm (about 65°C) absolute ethanol and dissolving the zinc chloride in the ionized water. The two solutions were then slowly mixed together with stirring and warming until approximately 70% of the solvents were evaporated. The mixtures were then formulated into a PEGO base to obtain a consistency suitable for topical application.

Mixtures 25 and 26 were prepared by dissolving the NDGA in a portion of the PEGO by stirring it while warming. The zinc chloride was dissolved in water and warmed while added to the remaining PEGO. The two solutions were then mixed while still warm and stirred until cooled to room temperature.

The compositions of the various NDGA and zinc chloride mixtures are given in Table 3 (all amounts are given in approximate weight/weight percents).

TABLE 3

Mixture	$ZnCl_2$	NDGA	Solvent	PEGO
21	40	10.1	15.1	34.6
22	30	7.4	11.2	51.2
23	44.8	11.2	17.2	27.3
24	16.8	4.2	6.5	72.4
25	27.5	6.9	18.3	47.2
26	6	33	21	40

EXAMPLE 4

The NDGA and zinc chloride mixtures of Example 3 were tested for their potential antitumor activity against B-16 and S-180 tumors grown in mice in accordance with the procedure previously described. The results are given in Table 4.

TABLE 4

B-16 Melanoma Study

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
21	10	0	0 (2876±1202)	100 (0)	100 (0)
21	10	1	31±10 (2876±1202)	90 (0)	100 (100)
21	10	0.6	7±2.3 (1366±676)	90 (0)	100 (80)
22	10	1	19±31 (1366±676)	40 (0)	60 (80)
22	10	8	199±234 (2505±1844)	30 (0)	100 (100)
23	9	3	23±48 (654±335)	77 (0)	100 (77)
23	9	47	512±372 (1091±547)	11 (0)	100 (80)
24	10	12	164±144 (1366±676)	11 (0)	90 (80)
24	10	63	1582±1749 (2505±1844)	12 (0)	80 (100)
25	10	0	0 (872±642)	100 (0)	100 (70)

TABLE 4 (Cont)

S-180 Solid Tumor Study

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
21	10	30	278±403 (934±656)	60 (10)	100 (100)
21	10	30	296±389 (997±421)	40 (0)	100 N/A
21	10	5	46±137 (997±421)	90 (0)	100 N/A
22	8	28	304±385 (1095±360)	37 (10)	100 (100)
22	10	108	1011±679 (934±656)	0 (10)	70 (100)
23	8	8	42±73 (550±184)	50 (0)	100 (100)
23	10	23	231±310 (997±421)	44 (0)	90 N/A
24	10	150	1398±762 (934±656)	10 (10)	100 (100)
24	10	12	164±144 (1366±676	11 (11)	90 (80)
26	5	132	937±973 (709±542)	0 (0)	80 (100)
26	5	98	697±500 (709±542)	0 (0)	80 (100)

The N/A designation means that no data are available.

EXAMPLE 5

Several different mixtures of butylated hydroxy-toluene (BHT), edetic or ethylenediaminetetraacetic acid (EDTA), NDGA and zinc chloride were formulated with a PEGO base. The mixtures were prepared by stirring together and warming a portion of the PEGO and all of the BHT and NDGA until a clear solution was obtained. The zinc chloride, which was weighed quickly to avoid increased weight of absorbed moisture, was dissolved in water with warming. Thereafter, the EDTA was added to the zinc chloride solution with stirring and warming until it was dissolved. At this point, the zinc chloride - EDTA solution of mixtures 35, 36, 37 and 38 were titrated with a 50 wt/wt percent solution of sodium hydroxide to obtain pH's of 4.5, 4.5, 2.0 and 3.0, respectively. The remainder of the PEGO 400 was added, with stirring, to the zinc chloride and EDTA until a clear solution was obtained. The resulting two solutions were then mixed together, while still warm, and stirred until cooled to room temperature.

The compositions of the mixtures are described in Table 5.

TABLE 5

Mixture	$ZnCl_2$	NDGA (meso)	EDTA	BHT	H_2O	PEGO 400	pH
27	27.5	6.9[2]	14.7	4.6	18.3	28[1]	--
28	27.5	6.9[2]	14.7	4.6	18.3	28	--
29	27.5	6.9	14.7	4.6	18.3	28	--
30	29.8	4.6	14.8	4.6	18.3	27.9	--
31	29.8	4.6	16.9[3]	4.6	16.1	27.9	--
32	13.8	3.5	7.4	2.3	9.1	63.9	--
33	5.5	1.4	3	0.9	3.7	85.5	--
34[4]	16.4	6.9	8.6	4.7	18	28	--
35[5]	23.6	6.9	12.7	4.7	20	28	4.5
36[6]	19.8	6.9	10.6	4.7	19.5	28	4.5
37[7]	25.4	3.9	14.3[3]	3.9	27.3	23.8	2.0
38[8]	12.7	2	7.1[3]	2	13.6	61.9	3.0

[1]PEGO had molecular weight of 200.
[2]NDGA is d,l-NDGA
[3]Disodium salt of EDTA
[4]NaOH constituted about 17% (wt/wt) of the mixture
[5]NaOH constituted about 4% (wt/wt) of the mixture
[6]NaOH constituted about 9.5% (wt/wt) of the mixture
[7]NaOH constituted about 1.4% (wt/wt) of the mixture
[8]NaOH constituted about 0.7% (wt/wt) of the mixture

EXAMPLE 6

The mixtures of Example 5 were tested for their potential antitumor activity against B-16 melanoma grown in mice in accordance with the procedure previously described. The results are given in Table 6.

TABLE 6

Mixture	n	T/C[1]	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
27	5	8	100±139 (1366±676)	60 (0)	100 (80)
28	7	2	15±53 (982±530)	80 (0)	71 (100)
29	7	5	52±66 (982±530)	43 (0)	100 (100)
29	10	1	16±45 (1805±968)	87 (0)	80 (80)
29	10	0	0 (696±646)	90 (0)	90 (80)
29	10**	32	176±199 (554±262)	30 (0)	80 (80)
29	10	2	12±38 (711±286)	90 (0)	100 (100)
30	10	4	26±79 (696±646)	90 (0)	100 (80)
30	10	0	0 (711±286)	100 (0)	100 (100)
31	10	4	31±91 (696±646)	80 (0)	90 (80)
32	9*	0	0	78 (0)	78 (70)
32	8*	0	0 (522±350)	88 (0)	88 (88)
32	10*	0	0 (1733±2254)	70 (0)	70 (80)
33	8*	19	106±202 (522±350)	75 (0)	100 (88)

TABLE 6 (Cont)

Mixture	n	T/C[1]	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
34	10**	78	435±155 (544±262)	10 (0)	90 (80)
34	10	92	651±354 (711±286)	0 (0)	100 (100)
35	10**	23	132±134 (544±262)	20 (0)	100 (80)
35	10	9	64±149 (711±286)	80 (0)	100 (100)
36	10**	85	469±544 (544±262)	0 (0)	90 (80)
36	10	133	948±886 (711±286)	10 (0)	100 (100)
37	6	260	1112±155 (428±463)	- -	- -
38	10*	0.1	2±5 (1733±2254)	80 (0)	90 (80)

*Animals treated once via 0.05 ml intratumor injection at day 6 after tumor innoculation.

**Animals treated topically once at day 6 after tumor innoculation.

[1]T/C ratio was calculated at day 21-25 for all experiments.

EXAMPLE 7

Mixtures of zinc chloride, EDTA and NDGA were prepared and formulated in a PEGO base. The mixtures were prepared by dissolving the NDGA and EDTA in a portion of the PEGO base by warming and stirring until dissolved. The zinc chloride was dissolved in water and warmed. The warm zinc chloride solution was added to the warm PEGO containing the NDGA and EDTA and stirred until cooled to room temperature. The composition of the mixtures is given in approximate weight/weight percentage as set forth in Table 7.

TABLE 7

Mixture	$ZnCl_2$	NDGA	EDTA	H_2O	PEGO
53	27.5	6.9	14.7	18.3	32.6
54	28	6.8[1]	14.7	18.2	32.9
55	16.4	6.9	8.6	18[2]	32.2

[1] d,l-NDGA was utilized.

[2] 50 w/w% NaOH was added to $ZnCl_2$ solution until a pH of about 4.5 was obtained. NaOH equaled about 17 w/w% of final mixture.

EXAMPLE 8

The mixtures of Example 7 were tested for their potential antitumor activities against B-16 melanomas grown in mice in accordance with the procedure previously described. The results are given in Table 8.

TABLE 8

B-16

Mixture	n	T/C**	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
53	10*	0	0 (575±270)	80 (0)	80 (60)
53	10	8	51±118 (711±286)	70 (0)	100 (100)
54	10	0	0 (711±286)	60 (0)	100 (100)
55	10	73	522±366 (711±286)	10 (0)	100 (100)

* - Animals were treated once via a 0.05 ml intratumor injection at day 6 after tumor.

** - T/C ratio calculated at day 24 except for Mixture 53 which was calculated at day 21.

EXAMPLE 9

A mixture comprised of BHT, EDTA, NDGA and zinc iodide was prepared and formulated into a PEGO base.

Zinc iodide was dissolved in warm water and EDTA was added with stirring until it was almost completely dissolved and turned yellow. BHT and NDGA were added to warm PEGO and this mixture was then added to the still warm zinc solution. Rapid stirring of the combination of these two mixtures in an ice bath resulted in a thick brown product.

The composition of the product in approximate w/w% is set forth in Table 9.

TABLE 9

ZnI_2	-	27.5%
NDGA	-	6.9%
EDTA	-	14.7%
BHT	-	4.6%
H_2O	-	18.3%
PEGO	-	28%

EXAMPLE 10

The mixture of Example 9 was tested for its anti-tumor activity against B-16 melanomas grown in mice in accordance with the procedure previously described. The results are given in Table 10.

TABLE 10

n	T/C @ Day 24	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
10	48	299±260 (617±358)	20 (0)	90 (90)

EXAMPLE 11

Mixtures of zinc chloride and BHT; zinc chloride, EDTA and BHT; zinc chloride and EDTA; zinc chloride, NDGA and BHT; and zinc chloride and ascorbic acid were formulated into PEGO bases.

The method of formulation was as follows: anhydrous zinc chloride was weighed quickly to avoid increased weight of absorbed moisture, placed into a beaker with water warmed in a water bath at 70-80°C and stirred until dissolved. If EDTA was a component, it was added with stirring to the warm zinc mixture until dissolved. When BHT or NDGA were included in a mixture, either or both were added to PEGO warmed in a water bath at 70-80°C and stirred until a clear solution was obtained. The two solutions were then mixed while still warm and stirred until cooled to room temperature and then refrigerated.

The zinc chloride, ascorbic acid solution was prepared by dissolving a measured quantity of each component in water, combining the two mixtures, then adding ethyl alcohol and PEGO. The compositions of the mixtures are given in Table 11.

TABLE 11

Mixture	$ZnCl_2$	NDGA	Ascorbic Acid	EDTA	BHT	H_2O	EtOH	PEGO
56	27.5	-	-	-	4.6	18.4	-	49.5
57	14.6	-	2.4	-	-	24.3	48.6	10
58	27.5	-	-	14.7	-	18.3	-	39.4
59	13.8	-	-	7.4	-	9.2	-	69.6
60	27.5	6.9	-	-	4.6	18.4	-	42.7
61	27.5			14.7	4.6	18.3	-	34.9

EXAMPLE 12

The mixtures of Example 11 were tested for their potential antitumor activity against B-16 melanoma grown in mice in accordance with the procedure previously described. The results are given in Table 12.

TABLE 12

B-16

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
56	10*	0	0 (872±642)	60 (0)	60 (70)
56	10	12	142±313 (1179±1041)	70 (0)	100 (60)
57	9	52	568±383 (1091±547)	11 (0)	100 (80)
57	9	13	86±114 (654±335)	44 (0)	100 (77)
58	10	1	22±59 (1805±968)	86 (0)	70 (80)
58	10*	0	0 (575±270)	30 (0)	30 (60)
59	8*	0	0 (872±642)	100 (0)	100 (70)
60	10	5	58±106 (1179±1041)	50 (0)	80 (60)
61	10	3	25±57 (804±559)	70 (0)	100 (100)
61	10	13	85±150 (644±342)	62 (0)	100 (70)
61	10	0	0 (1179±1041)	92 (0)	92 (60)

* - Animals treated once in via 0.05 ml intra tumor injection on day 6 or 7 after tumor innoculation.

EXAMPLE 13

Several mixtures wherein the zinc chloride was used in addition to another metal compound, or the zinc chloride was replaced by another metal compound were prepared.

Compositions of the mixtures as weight percent of the total composition are given in Table 13.

Mixtures 62, 63 and 68 were prepared according to the following method. NDGA was dissolved with stirring in warm (65°C) ethanol and quercetin was added with stirring until the solution was clear. Deionized water in which the sodium ascorbate had been dissolved was added dropwise with stirring. Then the metal salt was added and the stirring continued until the salt was dissolved. The solution was evaporated to dryness in a vacuum and these stored in an air-tight vial until mixed with the necessary amount of PEGO.

Mixture 64 was prepared by dissolving the NDGA in a minimal amount of ethanol. The cadmium salt and ascorbate were dissolved in deionized water and added to the NDGA solution dropwise with stirring and warmed to 65°C to effect solution. The mixture was evaporated to about 130% dry weight and formulated with the PEGO base.

For mixture 65, the salt was dissolved in water and mixed with PEGO base.

Mixture 66 was prepared by dissolving the NDGA, $ZnCl_2$ and $CuCl_2$ $2H_2O$ in ethanol. The copper caused oxidation of NDGA and a precipitate. The ethanol was evaporated, and PEGO was added to the product.

Mixture 67 was prepared in the same manner as 66, except that the cadmium salt was added in ethanol and water. A precipitate formed on evaporation and was mixed with PEGO. The product changed in consistency depending upon the temperature.

TABLE 13

Mixture	Salt	NDGA	Quercetin	Sodium Ascorbate	Water	PEGO	$ZnCl_2$
62	Cu $Cl_2.2H_2O$ 34.8	7	7	4.7	--	46.5	--
63	Cu Cl_2 20.9	7	7	4.7	--	60.4	--
64	Cd Cl_2 21.8	3.3	--	2.2	65.5	7.3	--
65	Cd Cl_2 53	--	--	--	15.9	30.9	--
66	Cu $Cl_2.2H_2O$ 31.6	12.7	--	--	--	30.4	25.3
67	Cd $Cl_2.2\ 1/2\ H_2O$ 55.3	8.3	--	--	--	19.9	16.6
68	Mn $Cl_2.4H_2O$ 29.5	5	5	3.4	--	56.8	--

EXAMPLE 14

The mixtures of Example 13 were tested for their potential antitumor activity against Sarcoma-180 tumors grown in mice in accordance with the procedures previously described. Test results are given in Table 14.

TABLE 14

S-180

Compound	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
62	5	51	343±444 (670±362)	40 (0)	100 (100)
63	5	100	675±247 (670±362)	0 (0)	100 (100)
64	9	0	0 (773±419)	100 (0)	100 (100)
64	10	43	530±616 (1240±521)	0 (0)	40 (100)
65	9	7	53±91 (773±419)	78 (0)	100 (100)
65	10	83	1032±713 (1240±521)	0 (0)	90 (100)
66	10	41	592±488 (1435±512)	30 (0)	100 (80)
67	9	20	281±439 (1435±512)	33 (0)	100 (80)
68	5	85	1375±638 (1609±883)	0 (0)	100 (100)

EXAMPLE 15

Six different mixtures were prepared having the compositions shown in Table 15. Mixtures A-E were prepared by mixing the quercetin, NDGA and sodium hydroxide crystals together in approximately 300-400 milliliters of water. The mixture was heated to approximately 60°C and it took approximately 1/2 to 1 hour for the mixture to dissolve while being stirred constantly. The resulting solution had a red-orange color. In those mixtures where zinc chloride and/or sodium ascorbate were added, they were first dissolved in approximately 300 milliliters of water. Then the two solutions were mixed together with stirring to form the mixtures. Immediately upon their intermixing a cottage cheese mixture was formed which, with continued stirring, became a slurry. The slurry was then roto-evaporated to dryness.

Mixture F was prepared by putting the zinc chloride into solution in water and then adding sodium ascorbate to zinc chloride solution. The NDGA was added to the zinc chloride and sodium ascorbate aqueous solution. The solution was a little cloudy indicating that not all of the NDGA was in solution. Approximately 10 drops of concentrated sodium hydroxide (approximately 40%) were added and the solution cleared a little. The quercetin was then added slowly with constant stirring and another approximately 5 drops of concentrated sodium hydroxide was added dropwise. The solution turned yellow to slightly orange and a flocculant precipitate formed. The precipitate was then dried by evaporation in a rotovap at approximately 50-60°C. Each of the mixtures was formulated into a PEGO (polyethylene glycol) base.

The difference in the two techniques in the preparation of the mixtures is thought to result in all of the zinc being complexed with other ingredients of the mixtures A-E. Whereas, the use of less sodium hydroxide

in mixture F resulted in there being some free zinc chloride as well as free NDGA and free quercetin.

TABLE 15

	Mixture					
Components(gm)	A	B	C	D	E	F
NDGA	1.5	1.5	1.5	1.5	1.5	1.5
Quercetin	1.5	2.5	2.0	2.0	2.0	1.5
Na ascorbate	1.0	1.0	1.0	1.0	0.0	1.0
$ZnCl_2$	3.0	5.0	5.5	0.0	0.0	15.0
PEG0[2]	3.0	3.0	3.0	8.0	9.5	6.0

EXAMPLE 16

The mixtures of Example 15, in addition to controls, were tested against xenografts of the transplanted human lung squamous cell carcinoma, LX-1, and human breast adenocarcinoma, MX-1 in athymic (nude) mice of BALB/c background. Each animal was inoculated intradermally on the dorsum near the nape of the neck with 0.05 ml. of a LX-1 or MX-1 tumor homogenate. Tumor weights, in milligrams, were calculated from the measurement of the length (L), width (W) and height (H), in millimeters of the tumors using the formula (LxWxH)/2. The animals were randomized in groups to ensure representation of smaller and larger tumors.

Topical treatment of the tumors was utilized and to assure penetration of the mixtures, the tumors were punctured with an 18 gauge (1-1/2 inch) needle to a depth slightly above the bevel (3/16 inch). The number of punctures varied from 8 to 12 depending on the size of the tumor.

In Experiment 1, the animals were treated twice, 18 and 19 days after LX-1 tumor inoculation. The animals of Experiment 2 were treated once at day 25 after LX-1 tumor inoculation. The animals of Experiment 3 were treated once at day 23 after MX-1 tumor inoculation.

The results of Experiments 1, 2 and 3 are given below in Tables 16-A, 16-B and 16-C.

In the tables, the mean delta tumor weight is the difference in mean tumor weight between the day specified and the mean tumor weight on the day of treatment. The tumor growth inhibition (positive or zero mean delta tumor weight) is expressed as a %T/C value calculated from the average test delta TW/average control delta TW and may be positive or zero. Tumor regression (negative delta TW) is expressed directly as a percentage of the test change in tumor weight to the initial tumor weight and is preceded by an R. For each experiment the groups of mice treated with mixtures A-F are compared with the group of mice treated with the PEGO control.

TABLE 16-A

LX-1 Double Treatment

Test or Control Article	DAY OF FIRST TREATMENT No. of Mice	Tumor Weight Range	Mean Tumor Weight	15 DAYS AFTER 2ND TREATMENT No. of Mice	Mean Tumor Weight	Mean delta Tumor Weight	%T/C
A	8	100-567	310	7	810	+500	61
B	8	162-550	306	7	796	+490	60
C	8	144-550	305	7	912	+607	74
D	8	126-567	295	6	1366	+1071	131
E	8	100-550	298	8	512	+215	26
F	8	100-527	277	4	156	-121	R 44
Untreated Control	16	144-486	284	15	1009	+725	-
PEGO Control	8	172-446	300	7	1114	+814	-

TABLE 16-B

LX-1 Single Treatment

	DAY OF FIRST TREATMENT			15 DAYS AFTER TREATMENT			
Test or Control Article	No. of Mice	Tumor Weight Range	Mean Tumor Weight	No. of Mice	Mean Tumor Weight	Mean delta Tumor Weight	%T/C
F	8	72-220	151	5	141	-10	R 6%
PEGO Control	4	60-216	143	4	393	+250	-

THIRTY-SIX DAYS AFTER TREATMENT			
No. of Mice	Mean Tumor Weight	Mean delta Tumor Weight	%T/C
2	210	+59	7
1	952	+809	-

TABLE 16-C

MX-1 Single Treatment

Test or Control Article	DAY OF FIRST TREATMENT No. of Mice	Tumor Weight Range	Mean Tumor Weight	15 DAYS AFTER TREATMENT No. of Mice	Mean Tumor Weight	Mean delta Tumor Weight	%T/C
A	7	126-624	257	7	1571	+1314	97
B	6	49-616	288	5	1517	+1229	91
C	6	98-396	220	6	1175	+955	70
D	6	150-385	282	4	1485	+1203	89
E	7	105-484	257	7	2314	+2057	152
F	7	84-546	243	1	686	+443	32
Untreated Control	7	132-702	293	6	1642	+1349	-
PEGO Control	6	84-405	224	5	1582	+1358	-

EXAMPLE 17

To 36.7 grams of powdered Larrea divaricata were added 24.5 grams of powdered rosehips and the mixture was blended in a blender for 5 minutes. The blended mixture was then mixed with 100 milliliters of an aqueous solution containing 185.9 grams zinc chloride to form a paste. The paste was allowed to stand at room temperature for 24 hours. Thereafter, it was stirred and then placed in a screw-capped glass container. The container was placed in a humidified oven at 40°C for 5 days. This incubated paste was then suspended in 500 milliliters of triple distilled water and shaken at room temperature for 24 hours on a reciprocating shaker. The zinc chloride extract solution was then evaporated to near dryness on a rotary evaporator at 90°C under reduced pressure. A sufficient quantity of this dried zinc chloride extract was added to 120 grams of an ointment base consisting of 10% (w/w) stearyl alcohol and 90% (w/w) polyethylene glycol to obtain an ointment containing 70% (w/w) of the extract.

EXAMPLE 18

A sufficient quanitity of the paste of Example 17 was added to sterile deionized water to obtain a concentration of 10 grams per 100 milliliters of water. The aqueous mixture was thoroughly shaken for one hour on a reciprocating shaker, then the aqueous suspension was filtered through Whatman #1 filter paper in a Buchner funnel. The filtrate, an aqueous suspension, was used to irrigate wounds in the treatment of osteomyelitis.

EXAMPLE 19

Five selected human patients with osteomyelitis of duration of from several months to several years were treated topically with the solution of Example 18 and/or the paste of Example 17. In all instances, the osteomy-

elitis had been unresponsive to conventional treatment, and upon the application of the preparation, the patients received no other conventional therapy except as indicated. In some cases, the wounds were debrided, prior to the application of the preparation. Upon application of the preparation, most patients experienced pain and a burning sensation over the area which had been treated and some patients additionally experienced swelling and inflamation. One patient experienced severe nausea after an application of the preparation.

Summaries, histories, and treatment are given below in Table 19. With respect to patient one, the disease process was so extensive that prior to treatment, a partial amputation of his foot was indicated. With respect to patient four, the disease process was so extensive as to cause the exposure of the extensor tendons which normally necessitates their cutting. Moreover, as a result of the destruction of the bones of the ankle and foot, the possibility of an ankle fusion was considered; however, neither of these procedures was required as the patient became ambulatory without the assistance of either a cane or crutches within six months of the beginning of the treatment with the preparation.

TABLE 19

Patient	Diagonosis	Culture	Previous Treatment	Duration of Condition	Number of Treatments	Time Required for Healing of Lesion
1 (62 year old male)	Chronic diabetic ulcer of left foot with osteo-myelitis extending down to the metatarsal head capsule, involving the flexor tendon of the fourth toe	Hemolytic Staphylcoccus aureus coagulase positive	Antibiotics with no response	Several months	2:13 days apart	1-1/2 months
2 (59 year old male)	Chronic ulceration of lateral aspect of the proximal fibula	Staphylcoccus aureus coagulase positive	Multiple skin graftings; multiple antibiotics	Several years	3:19 and 23 days apart	3 months
3 (63 year old male)	Chronic osteo-myelitis of left ankle and distal tibia	Hemolytic Staphylcoccus aureus coagulas positive	Recent treatment with Betadine soaks	35 years	4:over a 3-month period (first two with the solution and last two with the paste)	9-1/2 months for complete recovery

TABLE 19 (Cont)

Patient	Diagonosis	Culture	Previous Treatment	Duration of Condition	Number of Treatments	Time Required for Healing of Lesion
4 (70 year old female)	Ulcer of the left foot with necrosis, drainage, destruction of the bones of the foot and ankle initiated by a bite from a brown recluse spider	Hemolytic Staphylcoccus aureus coagulase positive	Antibiotics and soaks	7 months	2:5 days apart	1-1/2 months for lesion; after 6 months able to walk without crutches
5 (68 year old male)	Stasis ulcers of lower left extremity due to circulatory impairment	-	Steroid cream and ointment	unknown	2:9 days apart. Treated with a diuretic and soaks were applied to the area to reduce swelling apparently caused by the treatment.	2-1/2 months

EXAMPLE 20

Fifteen older dogs having perianal adenomas were treated topically with the ointment of Example 17 having a strength of 55% (w/w). The normal treatment for such a condition is surgery; however, these older dogs were poor surgical risks. The tumor of each dog was biopsied and the ointment was applied topically into the biopsied incision. The duration of treatment varied depending upon the severity of the adenoma. Dogs with simple circumscribed adenomas required only one treatment. The dogs with more advanced adenomas generally required more than one treatment which were given three to five days apart. The treatment was successful in thirteen of the fifteen dogs. The treatment was not successful in two of the dogs which had extremely advanced cases of perianal adenomas.

EXAMPLE 21

An incubated paste of rosehips, zinc chloride and Larrea divaricata prepared in accordance with the method of Example 17 was placed into gelatin capsules such that each capsule contained 200 mg of the paste. A patient with glioastrocytoma was treated orally with these capsules. Prior to this treatment the patient had a resistant tumor which displaced the cranium and protruded from the right lateral aspect of the skull; the protrusion measured 7 x 7 mm. The patient received 200 mg oral doses four times a day for a total daily dose of 800 mg. Observable and subjective improvement occurred within seven days; in 71 days the tumor had become cystic and lysed. The protuberance of the skull was reduced to near normal dimensions by repeated aspirations of the clear amber cystic tumor fluid. The patient has been maintained on the 200 mg capsules given four times daily and has remained symptom free for over 18 months.

Examples 22 through 35 describe the results of an investigation into the antineoplastic activity of a number of compositions in a series of experiments against human cancer xenografts implanted in athymic Balb/C (nude) mice. The mice were maintained under special research conditions which included positive laminar flow ventilation and sterilized food and water. Animals were identified with standard ear tags.

When the mice were six to eight weeks old, a fragment, weighing approximately 25 mg., of a human cancer was implanted subcutaneously in the left flank under anesthesia and the incision was closed and allowed to heal. The tumors were allowed to grow until they reached an approximate size of between 25 and 100 mm^2 (length x width). The approximate weight of the tumor was determined by measurement with vernier calipers, according to the formula:

$$\frac{A \times B \times C}{2} = \text{Weight (mg.)}$$

A = length in mm.,
B = width in mm., and
C = height in mm.

The human tumors investigated were breast adenocarcinoma - MX-1, lung squamous cell carcinoma - LX-1, renal cell cancer, a brain cancer (glioma), a melanoma, and a colon cancer.

Five mice were treated with the test composition for most experiments. The mice were anesthetized and given a single 0.05 ml. or 0.10 ml. intratumor injection of the test composition using a 23 or 27 gauge needle. The test composition administered in the following Examples is 0.05 ml., unless otherwise indicated. The day of treatment was considered as Day 1.

The animals were visually inspected, palpated, and their tumors were measured at intervals throughout the experiment. Often after treatment, necrotic or cratered skin tissue resulted. The size of this necrotic area was measured as length times width and the height was recorded

as zero. After the cratered area healed somewhat, it was possible to palpate the area and note whether a tumor remained, or regrew.

The mice were observed for a total of approximately 60 days, and were then sacrificed. The following categories of animal response were decided upon to summarize and report treatment results.

Tumor Free at 60 Days - indicates an animal that has survived the entire 60 day test period and is free of tumor at the end of that time.

Tumor at Death - the animal has a tumor when it dies or is sacrificed. The end point may occur before the 60 day period is complete.

Premature Death - the animal fails to survive the full 60 days having died of unknown causes, or is sacrificed when obviously ill to avoid cannibalization. The category does not include animals sacrificed due to massive tumor growth, those animals dying of anesthetic overdose or those which are actually cannibalized by their cage mates.

Tumor Recurrence - includes animals in which the tumor reappeared after an earlier tumor-free period. These are animals in which the original eschar has resolved, and palpation has indicated an absence of a tumor for some period; however the tumor has now regrown.

Animals may be reported in more than one category. For example, an animal found dead in its cage but having a tumor would appear in both the "Premature Death" and "Tumor at Death" categories.

Where the procedure of the test varied from this general protocol, the deviations are indicated in each specific example.

EXAMPLE 22

Test compositions were prepared according to the following general method. A stock solution of zinc chloride was prepared by dissolving a measured amount in water with heating and stirring - NDGA, or its analog desmethyl NDGA, was dissolved in PEGO 400 with heating and stirring. The two components were mixed together and allowed to cool with vigorous stirring. Additional PEGO 400 was added to further dilute the test compositions to achieve the approximate concentrations in wt/wt % given below. More than one stock solution was prepared according to the above-described procedure; the various test compositions were tested at different times.

The formulations were then tested according to the protocol previously described in groups of five athymic mice implanted with the MX-1 strain of human breast adenocarcinoma. Results are given in Table 22.

TABLE 22

Test Group	% NDGA	% $ZnCl_2$	Tumor Free 60 Days	Pre-mature Death	Tumor at Death	Tumor Recur-rence
1	1.0	0.5	2	0	3	4
2	1.0	1.0	3	0	2	2
3	1.0	2.0	5	0	0	0
4	2.0	0.5	4	0	1	1
5	2.0	1.0	0	0	5	5
6	2.0	2.0	4	0	1	1
7	3.0	0.5	2	0	3	3
8	3.0	1.0	0	0	5	5
9	3.0	2.0	4	0	1	1
10*	1.0	1.0	1	0	4	4
11*	2.0	1.0	3	0	2	2
12*	3.0	1.0	2	0	3	3
13	1.1	1.38	4	0	1	0
14	2.2	1.38	2	0	3	0
15	4.4	1.38	2	1	1	0
16	4.4	0.69	4	0	1	0
17	8.8	0.69	5	0	0	0
18	17.6	0.69	4	0	1	0
19	4.4 (desmethyl NDGA)	0.69	3	0	1	0
20	8.8 (desmethyl NDGA)	0.69	2	0	3	1
21	17.6 (desmethyl NDGA)	0.69	3	1	2	0
Control	--	--	0	0	5	0

* 0.10 of test composition administered.

EXAMPLE 23

For Examples 23 through 25 the test mixtures used were prepared by the following general method. In each case, any missing component was replaced by an equal amount of PEGO 400. If the general method differs, that difference is noted in the specific example.

When NDGA, or its analogs desmethyl NDGA or d,l NDGA, is included in a mixture, it is dissolved in PEGO 400 by warming and stirring. If butylated hydroxytoluene (BHT), is included, it is added to the PEGO-NDGA mixture and put into solution with warming and stirring. A second mixture is prepared by dissolving zinc chloride in water with warming and stirring. If used, edetic acid or ethylenediaminetetraacetic acid (EDTA), is added to the aqueous solution and dissolved with additional stirring. The two solutions are then combined while still warm and stirring is continued until the product cools to room temperature.

The two mixtures given below were prepared by the method described above and final compositions were as follows:

Group A - 4.0 w/w% zinc chloride
2.1 w/w% NDGA
0.97 w/w% EDTA
2.6 w/w% water
90.4 w/w% PEGO 400

Group B - 4.2 w/w% $ZnCl_2$
2.2 w/w% EDTA
1.0 w/w% desmethyl NDGA
2.9 w/w% water
89.7 w/w% PEGO 400

Control - polyethylene glycol 400

Additional mixtures of varying concentrations of zinc chloride, NDGA and EDTA were also prepared as described above and the pH of the mixture was measured. All of the mixtures were tested against human breast adenocarcinoma, MX-1, grown in five athymic Balb/c mice following the procedure previously described.

Test results are given in Table 23.

TABLE 23

Test Group	% NDGA	% $ZnCl_2$	% EDTA	pH	Free at 60 Days	Pre-mature Death	Tumor at Death	Tumor Recur-rence
A	2.1	4.0	0.97	--	3	0	2	0
B	1.0[2]	4.2	2.2	--	6	0	0	0
Controls[1]	--	--	--	--	0	0	6	0
1*	2.0	1.0	0.5	2.29	3	0	0	2
2*	2.0	1.0	1.0	2.07	3	0	2	2
3*	2.0	1.0	2.0	1.72	3	0	2	2
4*	2.0	1.0	--	3.16	4	0	0	1
5*	--	--	0.5	3.35	0	0	5	0
6*	--	--	1.0	3.38	0	0	0	0
7*	--	--	1.5	3.32	0	0	0	0
8*	--	--	2.0	3.34	0	0	0	0
9*	--	0.5	1.0	2.03	2	0	3	3
10*	--	1.0	1.0	1.85	5	0	0	0
11*	--	1.0	2.0	1.69	1	0	4	4
12*	--	1.0	--	3.24	1	0	4	4
13*	2.0	--	0.5	3.33	1	0	4	4
14*	2.0	--	1.0	3.32	0	0	4	1
15*	2.0	--	2.0	3.32	0	0	5	0
16*	2.0	--	--	3.48	0	0	5	1

1) 6 animals in test group.

2) desmethyl NDGA

* 0.10 of test composition administered.

EXAMPLE 24

Test materials of the compositions given below were prepared according to the general method described in Example 23 except that the composition of Group C was treated in the following manner. After the zinc chloride and EDTA were dissolved in the warm water, this test solution was titrated with a 50% solution of NaOH in water until the zinc solution reached pH 4.5. In all other respects preparation remained the same.

Group A -	0.6	w/w%	BHT
	2.1	w/w%	EDTA
	0.9	w/w%	NDGA
	3.9	w/w%	$ZnCl_2$
	2.6	w/w%	water
	89.9	w/w%	PEGO 400
Group B -	0.8	w/w%	BHT
	2.4	w/w%	EDTA
	1.1	w/w%	desmethyl-NDGA
	4.5	w/w%	$ZnCl_2$
	3.1	w/w%	water
	88.1	w/w%	PEGO 400
Group C -	0.7	w/w%	BHT
	1.2	w/w%	EDTA
	1.0	w/w%	NDGA
	2.3	w/w%	$ZnCl_2$
	2.4	w/w%	NaOH 50% solution
	2.5	w/w%	water
	90	w/w%	PEGO 400

Group D - 0.6 w/w% BHT
2.1 w/w% EDTA
0.9 w/w% NDGA (d,l form)
3.9 w/w% zinc chloride
2.6 w/w% water
90 w/w% PEGO 400

Controls - polyethylene glycol 400

The compositions were tested for antitumor activity in five athymic Balb/c mice implanted with human breast adenocarcinoma, MX-1, according to the protocol previously described. Test results are given in Table 24.

TABLE 24

Test Composition	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Group A	3	1	2	0
Group B	5	0	0	0
Group C[1]	3	0	3	2
Group D[1]	3	0[2]	2	1
Controls[1,3]	0	0	6	0
Controls[1,3]	0	1	5	0

1 - 6 animals in test group
2 - one animal died of anesthesia overdose
3 - tests performed at different times and more than one control group is reported

EXAMPLE 25

The following compositions were prepared according to the general method described in Example 23. The few changes in method are described after listing of test compositions.

Compositions of test materials were as follows:

Control: Polyethylene glycol (PEGO 400)

The mixtures of Groups A through E each had the following components in common:

0.6 w/w% BHT
2.1 w/w% EDTA
3.9 w/w% zinc chloride
2.6 w/w% water
90 w/w% PEGO 400

In addition to the above ingredients the mixtures of Group A-E contained the following ingredient:

Group A - 0.9 w/w%
Maracarb[1] Code No. DBO4G-215
Group B - 0.9 w/w% guaiacol
Group C - 0.9 w/w%
Kelig - 32[1] Code No. Y03H-2853
Group D - 0.9 w/w% of NDGA and the EDTA
was the disodium salt
Group E - 0.9 w/w% NDGA

The mixture of Group F consisted of only the following components:

Group F - 4.3 w/w% zinc chloride
2.3 w/w% water
93.4 w/w% PEGO

[1]Maracarb and Kelig-32 are trademarks for wood-derived products of American Can Company, Lignin Chemicals, American Lane, Greenwich, Connecticut 06830. The compositions are soluble in water and able to chelate metal ions.

In formulating the mixtures listed above, the lignins, Marcarb and Kelig-32 were dissolved in cold water to which was then added the $ZnCl_2$. The EDTA was the third component added to the aqueous solution and was warmed with stirring until dissolved, or in the case of

the Kelig-32 solution, until the EDTA was almost dissolved. The rest of the general procedure was followed, but the resulting product was then milled in an ink mill at 0.002".

The mixtures were tested for antitumor activity in five Balb/c mice implanted with human breast adenocarcinoma, MX-1, following the protocol hereinbefore described.

Test results are given in Table 25.

TABLE 25

Test Composition	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Group A	5	1	0	0
Group B[1]	6	2	0	0
Group C[2]	1	2	2	0
Group D	3	1	1	0
Group E[3]	2	0	0	0
Controls	0	0	5	0
Group F	2	2	1	0

1 - Test group contained 6 animals
2 - Test group contained 3 animals
3 - Test group contained 2 animals

EXAMPLE 26

Test compositions were prepared according to the following general method. The NDGA, BHT, and PEGO 400 were measured and mixed together with heating until melted and dissolved. The PEGO Base, consisting of 50% Pego 400, 45% Pego 3350 and 5% stearyl alcohol, was prepared by mixing and heating the components together in a separate container until they dissolved. $ZnCl_2$ and EDTA were dissolved in water with heating and stirring in a separate container. The ingredients in each of the

separate containers were added together in amounts needed to give the concentrations desired and allowed to cool with vigorous mixing. Any further dilution to achieve desired wt/wt % was achieved by adding Pego 400. When an ingredient was omitted from a particular composition, the amount of the missing ingredient was supplied by adding additional Pego 400.

Approximate wt/wt % of compositions utilized in this experiment are given below.

	Test Composition in wt/wt %		
Ingredient	1	2	3
$ZnCl_2$	4.3	4.3	4.3
Purified water	2.6	2.6	2.6
EDTA	-	2.1	2.1
NDGA	0.66	0.66	0.66
BHT	0	0.66	0
Pego 400	91	88.28	88.9
Pego Base	1.4	1.4	1.4

The test compositions were tested in five athymic mice implanted with human breast adenocarcinoma, MX-1. Results are given in Table 26.

TABLE 26

Test Composition	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
1	4	1	0	0
2	4	0	1	1
3	5	0	0	0

EXAMPLE 27

Two test compositions were prepared according to the general procedure previously described. However, in Composition 1 the zinc chloride was replaced by zinc iodide, and in Composition 2 the zinc chloride was

replaced by zinc bromide. Approximate concentrations of the ingredients are given below in wt/wt percent.

	Test Composition	
Ingredient	1	2
BHT	0.65	0.72
EDTA	2.1	2.3
NDGA	0.98	1.1
ZnI_2	3.9	-
$ZnBr_2$	-	4.3
H_2O	2.6	2.9
Pego Base	1.4	0
Pego 400	88.37	88.68

The two compositions were tested for antitumor activity against human breast adenocarcinoma, MX-1, grown in five athymic mice as previously described. The results are given in Table 27.

TABLE 27

Test Composition	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Re-currence
1	4	1	0	0
2	4	0	1	0

EXAMPLE 28

Test compositions containing a proprietary slow-release formula of KV-Pharmaceutical Co. and varying proportions of BHT, EDTA, NDGA and zinc chloride was prepared according to the general method described in Example 26. The proportions of BHT, EDTA, NDGA, and zinc chloride are shown in Table 28. The remaining ingredient in the test composition and the control comprised the proprietary compound. These preparations were tested for their effectiveness as antitumor agents against xenografts of the human breast adenocarcinoma, MX-1, grown in

athymic mice, by intratumor injection, according to the protocol previously described. Results are set forth in Table 28.

TABLE 28

Test Composition No.	BHT %	EDTA %	NDGA %	$ZnCl_2$ %	Tumor Free 60 days	Pre-mature Death	Tumor at Death	Tumor Recurrence
1	2.39	0.49	10	1.0	4	1	0	0
2	3.59	2.47	15	5.0	5	0	0	0
3	0.24	4.93	1.0	10	3	2	0	0
Control	0	0	0	0	2	0	3	0

EXAMPLE 29

A test composition was investigated for its anti-neoplastic activity against xenografts of the following human cancers: lung squamous cell carcinoma, LX-1; breast adenocarcinoma, MX-1; renal cell cancer; brain cancer (glioma); melanoma; and colon cancer. The test composition with the approximate wt/wt percentages given below was prepared according to the procedure previously described in Example 26. A control composition consisting of Pego 400 was also prepared.

Ingredient	Test Composition 1	Control
BHT	0.16	
EDTA	2.10	
NDGA	0.66	
$ZnCl_2$	4.26	
H_2O	2.62	
Pego Base	1.43	
Pego 400	88.77	100

The composition was then tested for its effect on human tumors of varying origin implanted in athymic mice as previously described. Generally, there were ten mice in each group tested with composition 1, and five mice in each group tested with Pego 400 control. Instances in which the number of mice varied are specifically indicated.

Results are given in Table 29.

TABLE 29

Tumor Type	Test Composition	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
LX-1 (lung)	1	8	0	2	0
	control	0	0	5	0
MX-1 (breast)	1	8	0	2	1
	control[1]	0	0	2	0
Renal	1[2]	8	1	1	0
	control	0	1	5	0
Glioma (Brain)	1[3]	6	0	0	0
	control[1]	0	0	2	0
Melanoma	1[4]	10	0	0	0
	control	0	0	5	0
Colon	1	8	1	2	0
	control	0	0	5	0

1. There were only two mice in this control group.

2. Eleven mice tested. One mouse died from anesthesia overdose the first day of test and was replaced with another mouse.

3. Six mice tested. One mouse dead in cage on Day 60.

4. In one mouse the crust did not heal until the last day of the test.

EXAMPLE 30

For Examples 30 through 32, a number of organic compounds were formulated into test compositions according to the following general method.

Zinc chloride was dissolved in Pego 400 to prepare a stock solution. The amount of organic compound required to give the final concentration given below was measured into a clean vial and Pego 400 was added with mixing until dissolved.

An amount of the $ZnCl_2$ stock solution was then measured and the two solutions were mixed to give a final concentration in each test composition of zinc chloride at 0.69 wt/wt % and each organic compound at a molar concentration equivalent to 4.4 wt/wt % of NDGA for compounds having two or more benzene rings, and equal to 8.8 wt/wt% NDGA for compounds having one or no benzene rings. Nitrogen gas was blown over the top of the vial, a teflon liner was inserted in the cap and the vial was sealed and frozen until testing.

The test compositions reported in Table 30 are identified in the table by the organic compound they include, and were tested for their effectiveness as antitumor agents against xenografts of the human breast adenocarcinoma, MX-1, grown in athymic mice, by intratumor injection according to the protocol previously described.

TABLE 30

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Pego Control	0	0	5	0
benzene	0	0	5	1
pyrocatechol	0	0	5	2
phenol	1	0	4	0
pyrogallol	0	0	5	0
resorcinol	0	1	5	0
hydroquinone	2	0	3	0
3,4-dihydroxy-cinnamic acid	1	0	4	0
cinnamic acid	2	0	3	1
3-isopropyl catechol	0	0	5	0
4-hydroxy-3-methoxy cinnamic acid	2	0	3	0
4-tertbutyl catechol	2	1	2	1
3,4-dihydroxy benzaldehyde	3	0	2	0
4-methyl catechol	1	2	2	1
4-ethyl resorcinol	3	0	2	1
o-dimethoxy benzene	0	1	4	1
N,3-(3,4-dihydroxy phenyl) propyl piperidine	0	0	5	0
guaiacol	0	1	4	0
desmethyl-N-tetramethyl ether	2	0	3	0
quinizarin	1	1	3	1
8-hydroxy quinoline	0	0	5	0
olivetol	3	0	2	0
2-tertbutylphenol	2	1	2	0
3-tertbutylphenol	5	0	0	0
4-tertbutylphenol	5	0	0	0
2,3-dimethylphenol	2	0	3	0
thymol	4	0	1	0
5-nitrovanillin	2	0	3	1
o-anisidine	1	0	4	0

TABLE 30 (Cont.)

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
purpurogallin trimethyl ether	1	0	4	2
vanillin	1	1	4	2
p-hydroxy-cinnamic acid	4	1	0	0
dihydroxynaphthalene	3	0	2	0
2,5-dihydroxy-p-benzoquinone	2	2	3	1
orcinol	2	0	3	0
pentafluorophenol	3	1	1	0
picric acid	4	1	0	0
3-(3,4-dimethoxy-phenyl) propylamine-N, N-acid . HCl	2	0	3	0
3,4-dimethoxy-phenylethanol	2	1	2	0
3,4-dimethoxy-acetophenone	1	0	4	0
3-(3,4-dimethoxy-phenyl) propylamine	3	1	2	0
2,3-dihydroxy-benzoic acid	1	1	3	0
3,4-dihydroxyhydro-cinnamic acid	1	0	4	0
3,4-dihydroxy-phenylacetic acid	3	0	2	0
3,4-dihydroxy-benzoic acid	3	0	2	2
3,4-dimethoxy-cinnamic acid	0	2	5	0
2,3,4-trihydroxy-acetophenone	0	0	5	0
2,3-dihydroxy-benzaldehyde	2	0	3	1
3,4-methylenedi-oxypropiophenone	2	0	3	1
dl-NDGA	5	0	0	0
NDGA tetraacetate	4	0	1	0

TABLE 30 (Cont.)

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
1,4-bis(3,4-dimethyleneoxyphenyl)butene	2	0	3	1
vanillin azine	1	0	4	0
syringaldazine	2	0	3	0
2,3-bis(3,4-dimethoxybenzoyl) butane	1	0	4	0
dihydroguaiaretic acid	4	0	1	0
norisoguaiacin	5	0	0	0
NDGA tetramethyl ether diol	3	0	2	0
2,5-bis(3,4-methylenedioxyphenyl)-3',4'-dimethylfuran	1	0	4	0
2',3',4',3,4-pentahydroxy-1,4-diphenylbutane	2	1	3	0
3',4',5',3,4-pentahydroxy-1,4-diphenylbutane	3	0	2	1
1-(3,4-dihydroxyphenyl),4-(2,5-dihydroxyphenyl)butane	5	0	0	0
$NDGA(Zn)_2$ complex[1]	0	0	5	0
$NDGA(Zn)_1$ complex[2]	1	1	3	0
$NDGA(Zn)_1$ complex[3]	1	0	4	1
$NDGA(ONa)_4$	5	0	0	0
1-(3,4-dihydroxyphenyl)-4-phenyl butane	3	1	1	0
1,4 bis(3,4-dimethoxyphenyl),2-3 methylfuran	0	0	5	0
NDGA	4	1	0	0

[1] 4.4% N - all Zn tied up
[2] 4.4% N - 2% free Zn
[3] 4.4% N - all Zn tied up

TABLE 30 (Cont.)

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
calcein blue	1	1	3	1
quercetin	1	0	4	0
ellegic acid	4	0	1	0
4-methylesculetin	1	0	4	0
flavanone	3	2	0	0
flavone	4	0	1	1
lauric acid	2	1	2	0
adipic acid	4	0	1	0
azelaic acid	5	0	0	0
oxydiacetic acid	5	0	0	0
1-naphthaldehyde	3	1	1	0
2-naphthaldehyde	3	0	2	0
epipodophyllotoxin	5	0	0	0
podophyllotoxin	0	5	5	0
epipodophyllotoxin glycoside	3	0	2	0
VP-16	5	0	0	0
VM-26	5	0	0	0
lauryl alcohol	4	0	1	0
chloranil	1	0	4	0
n-octyl cyanide	3	1	2	0
octyl aldehyde	4	0	1	0
NDGA propionate	4	0	1	1
NDGA-Zn_2 complex	1	0	4	0
NDGA-Zn complex	1	1	4	0
3,4-dihydroxy-benzylamine-HBr	3	0	2	0
2-aminophenol	2	0	3	0
1,6-bis-(3,4-dihydroxyphenyl)-hexane	3	1	1	1

TABLE 30 (Cont.)

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
mandelic acid	5	0	0	0
1,4-bis(3,4-methylene-dioxyphenyl)-2,3-dimethyl-butane-1-4-diketone	0	1	4	0
3-propyl catechol*	4	0	1	0
1-4-bis(dihydroxy, diethylcarbonyl-methoxyphenyl), 2,3-dimethylbutane	1	0	4	4
3-(2,3-dihydroxy-phenyl)propylamine-N-N-diacetic acid dimethyl ester	0	0	5	5
1-(3,5-dinitro-phenyl)-4-(3,4-dimethoxyphenyl)-butene-1	1	0	4	3
1,4-bis(3,4-dimethoxystyrl)-benzene	1	0	4	4
1,4-bis(3,4-di-hydroxyphenethyl)-benzene	2	0	3	3
beta-beta-bis(3,4-dihydroxyphenyl)-1,4-divinyl benzene	1	0	4	4
nordihydroguaiaretic acid diphenoxy acetic acid diethyl ester	1	0	4	4
nordihydroguaiaretic acid diphenoxy acetic acid triethyl ester	4	0	1	1
1-(3,4-diacetoxy-phenyl)-4-phenyl-buta-1,3-diene	3	0	2	2
nordihydroguaiaretic acid tetraethyl hemisuccinate	5	0	0	0

TABLE 30 (Cont.)

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
nordihydroguaiaretic acid glucoside tetraacetate	1	0	4	3
nordihydroguaiaretic acid glucoside	3	2	0	2
1-(3,4-dihydroxyphenyl)-4-phenylbutadiene	3	2	0	2
2,3-bis(3,4-dimethoxybenzylidene)-succinic acid	2	0	3	3
diethylstilbestrol	3	0	2	1
1-(3,5-ditrifluoromethylphenyl)-4-(3,4-dimethoxyphenyl)-butene-1	1	0	4	4
1-(3,4-dihydroxyphenyl)-4-(3,5-ditrifluoromethylphenyl)butane	3	0	2	2
ethyl 3,4-dihydroxybenzoate	2	0	3	3
2-(3,4-dimethoxybenzylidene)-succinic acid	3	0	2	2
2-(3,4-dimethoxybenzylidene)-succinic anhydride	1	0	4	4
para-para-desmethyl nordihydroguaiaretic acid*	0	0	5	0
methyl carbamate nordihydroguaiaretic acid	1	0	4	4

TABLE 30 (Cont.)

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
2,3-bis(3,4-dimethoxybenzylidine)succinic anhydride	0	0	5	3
nordihydroguaiaretic acid*	1	1	3	3
ortho-para-desmethyl nordihydroguaiaretic acid*	2	0	3	3
NDGA-tetra-propionate*	4	0	1	1

*0.10 of test compound administered

EXAMPLE 31

A number of organic compounds were formulated and tested as set forth in Example 30 with 0.69% zinc chloride, and without zinc chloride. As part of this comparative testing, some of the organic compounds included known anticancer agents. The results of the organic compounds with and without zinc chloride are set forth in Table 31.

TABLE 31

Organic Compound	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
NDGA (no Zn)	4	1	0	0
NDGA + Zn	4	1	0	0
3-tertbutylphenol (no Zn)	1	3	1	0
3-tertbutyl-phenol + Zn	5	0	0	0
4-tertbutyl-phenol (no Zn)	5	0	0	0
4-tertbutyl-phenol + Zn	5	0	0	0
p-hydroxycinnamic acid (no Zn)	1	0	4	0
p-hydroxycinnamic acid + Zn	4	1	0	0
norisoguaiacin (no Zn)	2	1	1	0
norisoguaiacin + Zn	5	0	0	0
dl-NDGA (no Zn)	4	0	1	0
dl-NDGA + Zn	5	0	0	0
azelaic acid (no Zn)	1	0	4	0
azelaic acid + Zn	5	0	0	0
1-(3,4-diacetoxy-phenyl)-4-phenyl-buta-1,3-diene (no Zn)	1	0	4	0
1-(3,4-diacetoxy-phenyl)-4-phenyl-buta-1,3-diene + Zn	3	0	2	2
1,4-bis(3,4-di-hydroxyphenethyl)-benzene (no Zn)	2	0	3	3
1,4-bis(3,4-di-hydroxyphenethyl)-benzene + Zn	2	0	3	3

EXAMPLE 32

A number of organic compounds were tested in varying concentrations for their antineoplastic effectiveness against xenografts of human breast adenocarcinoma, MX-1, and grown in groups of five athymic mice, according to the protocol previously described. All compounds contained 0.69% Zn. Results are set forth in Table 32.

TABLE 32

Organic Compound	Dose mg/ml	Tumor Free 60 Days	Pre-mature Death	Tumor at Death	Tumor Recur-rence
o,p-desmethyl NDGA	40.0	5	0	0	0
o,p-desmethyl NDGA	8.0	2	0	3	3
d,l-NDGA	44.0	5	0	0	0
d,l-NDGA	8.8	3	1	1	1
d,l-NDGA	1.8	1	0	4	4
NDGA-tetrapropionate	76.5	4	0	1	1
NDGA-tetrapropionate	15.0	4	0	1	1
NDGA-tetrapropionate	3.1	1	2	2	2
3-propyl catechol	44.0	4	0	1	0
3-propyl catechol	8.8	4	0	1	1
3-propyl catechol	5.0	1	0	4	4
acetoxyphenyl buta-diene	45.0	3	0	2	2
acetoxyphenyl buta-diene	9.0	1	0	4	4
1,6-bis(3,4-di-hydroxyphenyl) hexane	44.0	3	1	1	1
1,6-bis(3,4-di-hydroxyphenyl) hexane	8.8	2	0	3	3
1,6-bis(3,4-di-hydroxyphenyl) hexane	4.0	1	0	4	4
NDGA-tetraacetate	66.3	4	--	--	--
NDGA-tetraacetate	13.3	1	0	4	4
1-(3,4-dihydroxy-phenyl)-4-phenyl-butane	36.3	3	--	--	--
1-(3,4-dihydroxy-phenyl)-4-phenyl-butane	12.5	1	1	4	4
1,4-bis-(3,4-di-methoxyphenyl)-buta-1,4-diol	57.5	3	--	--	--
1,4-bis-(3,4-di-methoxyphenyl)-buta-1,4-diol	11.5	1	0	4	4
3',4',5',3,4-penta-hydroxy-1,4-di-phenylbutane	47.0	3	--	--	--
3',4',5',3,4-penta-hydroxy-1,4-di-phenylbutane	9.4	1	0	4	2

EXAMPLE 33

A solution of 4.6% NDGA in polyethylene glycol 400 was diluted 1:20 and 1:80 with polyethylene glycol 400, and injected intraperitoneally into nude mice, bearing transplanted human breast adenocarcinoma, MX-1, tumors. Four mice were injected with the 1:20 dilution, and three mice were injected with the 1:80 solution. All mice were sacrificed on day 19 due to large tumor size. No retardation of tumor growth was observed.

EXAMPLE 34

Various metal salt compositions were tested to determine the compositions' antineoplastic effectiveness against xenografts of human breast adenocarcinoma, MX-1, grown in groups of five athymic mice, according to the protocol previously described. The concentration of the various metal salts in the test compositions was 0.73% (wt/wt) metal salt and 1.0% (wt/wt) NDGA, in a PEGO 400 base. The results of these test compositions are summarized in Table 34.

TABLE 34

Test Compound*	Tumor Free 60 Days	Pre-mature Death	Tumor at Death	Tumor Recurrence
$ZnSO_4.7H_2O$-NDGA	2	0	3	3
$ZnBr_2$-NDGA	2	0	3	3
Zn Acetate.$2H_2O$-NDGA	2	0	3	3
$Zn(NO_3)_2.6H_2O$-NDGA	3	1	1	1
$ZnCO_3$-NDGA	1	0	4	0
$CaCl_2.2H_2O$-NDGA	0	1	5	2
$MnCl_2.4H_2O$-NDGA	0	0	5	1
CuCl-NDGA	1	0	4	3
$CuCl_2$-NDGA	0	4	1	1
$FeCl_2.H_2O$-NDGA	0	0	5	3
$FeCl_3$-NDGA	1	2	3	3
VCl_3-NDGA	1	0	4	4
$PtCl_2$-NDGA	0	0	5	0
$CoCl_2$-NDGA	0	1	4	4
$PtCl_4$-NDGA	1	2	2	4
$GaCl_3$-NDGA	0	0	5	0
$Zn(Gluconate)_2.3H_2O$-NDGA	0	0	5	4
$AlCl_3$-NDGA	0	0	5	1
$MgCl_2$-NDGA	0	0	5	0

* 0.10 ml of test composition administered

EXAMPLE 35

For each composition as set forth in Table 35, two mice without tumors were injected subcutaneously on the flank. Also, for each of the same compositions two mice, each having a transplanted human breast adenocarcinoma, MX-1 tumor, were injected subcutaneously on the flank opposite that bearing the tumor. The injection sites healed well without ulceration or scarring in all cases. The animals with tumors were sacrificed on day 22 due to large tumor size. No retardation of tumor growth was observed.

TABLE 35

Compound	No. of Mice with Tumor	Tumor at Death*	No. of Mice without Tumor	Healing observed
Control (PEGO)	2	2	2	4
1.0% $ZnCl_2$, 4.6% NDGA, 1.1% BHT, 0.49% EDTA	2	2	2	4
5.0% $ZnCl_2$, 4.6% NDGA, 1.1% BHT, 2.47% EDTA	2	2	2	4
10.0% $ZnCl_2$, 4.6% NDGA, 1.1% BHT, 4.93% EDTA	2	2	2	4

*These animals originally had tumors, and were injected on the contralateral flank from the tumor.

EXAMPLES 36-45

Examples 36 through 45 describe the results of the investigation of the antineoplastic activity of four compositions in clinical studies on patients and other studies.

Four compositions, suitable for topical application were prepared, containing zinc chloride ($ZnCl_2$), nordihydroguaiaretic acid (NDGA), edetic or ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), stearyl alcohol, purified water, polyethylene glycol having an average molecular weight of 400 (PEGO 400), and polyethylene glycol having an average molecular weight of 3350 (PEGO 3350). The compositions were prepared in the following manner: the purified water was placed in a clean glass container of suitable capacity, the water was heated to about 80-90°C with stirring, and zinc chloride was added to the heated water, continuing the stirring until the zinc chloride dissolved. The edetic acid was next slowly added with mixing until dissolved. In a separate glass container of suitable size, the polyethylene glycol 400 was heated to about 80-90°C with stirring, the NDGA was added thereto, then the BHT, and this mixture was added to the zinc chloride-edetic acid solution with stirring. The entire mixture was then cooled to about room temperature and passed through a number 3 roller mill until smooth. The polyethylene glycol 3350 was then heated to about 80-90°C in a suitable container and the milled ingredients added thereto with mixing.

The four compositions given below were prepared by the method described above and the final compositions in wt/wt % were as follows:

Composition	Compound A	B	C	D	E
zinc chloride	29.8	1.0	5.0	10.0	20.0
NDGA	4.6	4.6	4.6	4.6	--
EDTA	14.7	0.49	2.47	4.93	--
BHT	1.1	1.1	1.1	1.1	--
stearyl alcohol	0.5	0.5	0.5	0.5	0.5
H_2O	18.3	18.3	18.3	18.3	18.3
PEGO 400	26.4	26.4	26.4	26.4	26.4
PEGO 3350	4.5	4.5	4.5	4.5	4.5

The test compositions were tested in clinical studies for its antineoplastic activity on various cancers and diseases on the patients.

The protocol for administering the compositions is as follows: for Compound A, no pre-treatment or preparation of the lesion or surrounding skin was done prior to application of the compound. Prior to topically applying Compounds B, C, or D on basal cell epithelioma and actinic keratosis, the surface of the lesions were tape stripped prior to each application. Tape stripping involves pressing a sticky medical or surgical tape on the lesion site, then removing the tape. The test compound was then applied to the lesion. Following the application of the compound, some of the lesions were covered with a dermatological dressing. The test compound remained on the lesion for at least 48-72 hours. A second application of the compound was made 48-72 hours following the first application using the identical technique described above.

EXAMPLE 36

Fifty-seven patients with basal cell epithelioma were treated with Compound A, B, C or D. The test medication was applied directly to the lesion with a coating of approximately 2 mm thick and confined to the

visual margins of the lesion. The lesion was then covered with a dressing and the patient was advised against washing the treated area for a reasonable period of time as determined by the investigator. A visual examination and measurement of the lesion was performed at 3-4 day intervals. At the discretion of the investigator, a second application of the same test compound was applied after a minimum of seven (7) days following the initial treatment. To determine the effect of the test compound on the malignant neoplasm, an excisional biopsy was obtained 30 days after the initial treatment.

Compound A was used to treat eight human patients with basal cell epithelioma. No pretreatments or preparations of the lesion or surrounding skin were done prior to application of the composition.

Three patients, T.W., H.S., and J.H., with basal cell epithelioma on the forehead, back and shoulder, respectively, were treated with a single topical application of the composition. A crust formed in all three patients following treatment and the wound was well healed within 40 to 56 days in two patients, T.W. and H.S. Biopsies in these two patients were negative for basal cell epithelioma. In patient J.H., forty days following the single application of the composition, the wound remained crusty and the biopsy was positive for basal cell epithelioma.

Four additional patients with basal cell epithelioma on the head and face, were treated with 2 topical applications of the composition. In addition, J.H. was retreated with 2 applications of the composition. A crust formed in all patients and the wounds healed within 30 to 40 days. Punch biopsies in three of these patients were negative and biopsies in patient J.H. and in one additional patient were positive for basal cell epithelioma. In these patients, the second application of the composition was placed not less than 7 days following the first

application. Table 36 summarizes the results of the clinical studies on human patients with basal cell epithelioma.

TABLE 36

Negative Biopsies/Evaluable Lesions

Investigator	Compound			
	A	B	C	D
1	-	-	-	-
2	-	0/4	1/4	0/31
3	-	-	0/1	4/5*
4	-	-	0/1	-
5	10/12**	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	0/2	0/2	1/2
9	-	-	-	-
10	-	-	0/1	1/1
11	-	-	-	1/21
12	-	-	-	2/12
13	-	-	-	-
14	-	-	-	-
15	-	0/2	0/1	0/2
	10/12	0/8	1/10	9/27

* The 1 patient who did not have a negative biopsy had a few residual basal cells at 1 margin of the post treatment biopsy. Physician believes this area of malignant cells was outside the area treated with the test formulation.

** One (1) patient had 2 lesions treated sequentially.

EXAMPLE 37

Fifty-nine (59) human patients with actinic keratosis were treated with Compound B, C, or D. The test medication was applied directly to the lesion with a coating of approximately 2 mm and confined to the lesion margin. A dressing was applied to the lesion and the patient was advised against washing the treated area for a reasonable period of time. A visual examination and measurement of the lesion was performed 7 and 14 days following the initial treatment. At the discretion of the investigator, a second treatment with the same test compound was applied. In order to determine whether the test compound eradicated the premalignant neoplasm, a punch biopsy was obtained 30-60 days after the initial treatment. If the biopsy report was negative, the patient was examined every 6 months for a period of 12 months. If the biopsy continued to show evidence of actinic keratosis, the patient was withdrawn from the study and treated with conventional therapy. The results of the study are given in Table 37.

TABLE 37

Negative Biopsies/Evaluable Lesions

Investigator	Compound		
	B	C	D
1	-	-	-
2	1/4	0/4	2/4
3	-	-	1/1
4	-	-	-
5	-	-	-
6	3/4	2/4	2/4*
7	-	-	0/5
8	0/1	0/1	0/1
9	-	-	4/12
10	-	-	-
11	-	-	-
12	-	-	6/12
13	-	-	1/4*
14	-	-	-
15	-	-	-
	4/9	2/9	16/43

* One (1) patient had 2 lesions treated concurrently.

EXAMPLE 38

Two human patients with recurrent cutaneous chest wall adenocarcinoma of the breast were treated with Compound A. The compound was applied directly on the lesion with a thickness of approximately 2 mm and confined to the visual margins of the lesion. One (1) lesion was punctured with a needle to facilitate penetration. A dressing was applied to cover the treated lesion and the patient advised against washing the treated area for a reasonable period of time. A crust formed at each site of the test compound application and

the area of the lesion was well demarcated. The patient returned 1, 2, 3, 7 and 14 days following the initial treatment for a visual examination and measurement of the lesion. A second application of Compound A was applied to three (3) lesions. On the 14th day after the initial application, a biopsy was obtained to determine the effect of Compound A on the malignant neoplasm. If the tumor was completely eradicated, the patient was examined periodically for 12 months. If the biopsy continued to show evidence of recurrent cutaneous chest wall adenocarcinoma of the breast or if the lesion was not clinically improved by the 14th day after the initial treatment, the patient was withdrawn from the study.

TABLE 38

Negative Biopsies/Patient Treated

Investigator	Compound A
5	0/2
14	0/3
	0/5

EXAMPLE 39

Eleven cutaneous ulcers in eight human patients were treated with Compound C. If excessive necrotic material was present, debridement of non-viable and foreign material was performed either surgically or with wet-dry dressings prior to treatment.

The test compound was applied directly to the cutaneous ulcer in an amount sufficient to cover the visual margins of the ulcer. The treated ulcer was then covered with a loose dressing and the patient advised against washing the treated area for a reasonable period of time. A scab or crust was observed to form on the surface of the ulcer. Normally within two weeks the

crust had loosened to where it was sluffed off or could be readily removed. It was observed that granulation of the tissue in the ulcer had occurred in those ulcers which showed clinical improvement. A second treatment with Compound C was applied after removal of the crust. The patient was visually examined and the ulcer measured within two weeks after the initial treatment. Thereafter, the patient returned twice a month for two months for a visual examination and measurement of the ulcer. Of the eleven (11) treated lesions, seven (7) were clinically improved.

EXAMPLE 40

Six (6) Kaposi's sarcomas in human patients were treated with Compound A, which was applied directly to the lesion with a thickness of approximately 2 mm and confined to the visual margins of the lesion. The lesion was then covered with a dressing and the patient advised against washing the treated area for a reasonable period of time. The patient was visually examined 1, 2, 3, 7 and 14 days after the initial treatment. If possible, accurate measurements of the lesion were taken and recorded. A second application of Compound A was applied as deemed necessary. After 14 days, a biopsy was obtained if the lesion appeared clinically improved. If the biopsy continued to show evidence of Kaposi's sarcoma or if the lesion was not clinically improved by the 14th day after the initial treatment, the patient was withdrawn from the study. Due to the serious nature of the disease, the 14-day time period was arbitrarily chosen as the termination point in order to provide patients who had not clinically improved the opportunity to pursue other methods of treatment regardless of biopsy results. The short duration of the study may account for the results which indicate that the test medication had no effect on the six (6) patients.

EXAMPLE 41

Canine patients with various tumor lesions were treated with Compound A, B, C, or D. The animals were restrained from movement for two hours physically or with sedatives (e.g. 0.03 mg oxymorphone/lb.sq with atropine sulfate). After clipping, washing and measuring the tumor site, the skin surface was abraded until bleeding occurred. To enhance the penetration of the test compound for large or subdermal tumors, a 20 or 22 gauge needle was used to puncture the tumor. After blotting the skin dry of blood, the tumor site was covered with a 1-2 mm coating of the test compound extending 5 mm peripherially. After 2 hours, the compound was wiped off and the area gently cleansed. The test compound was applied up to three times within a two-week interval or until the tumor cleared. The results of the canine studies are given in Table 41.

TABLE 41

Canine Studies

Canine Patient No.	Lesion/Location	Compound	Number Treatments	Final Biopsy Report
1	Mast cell tumor on abdomen	A	2	Mast cell tumor*
2	Mast cell tumor on head	A	3	Small volume of mast cells, mostly fibrous connective tissue**
3	Squamous cell carcinoma on prepuce	A	3	No squamous cell carcinoma observed
4	Mammary adenoma	A	3	Insufficient tissue for histological examination. No visible lesion. Animal euthanized due to diabetic complications.
5	Perianal adenocarcinoma	A	3	No visible tumor. Histological examination reports disorganized and less differentiated areas of the neoplasm but still indicates adenocarcinoma.
6	Perianal adenoma	A	2	Probably normal tissue
7	Perianal adenoma	A	2	Probably normal tissue
8	Perianal adenoma	A	3	Complete response - mass completely regressed.

TABLE 41 (Cont.)

Canine Studies

Canine Patient No.	Lesion/Location	Compound	Number Treatments	Final Biopsy Report
9	Perianal adenoma	A	3	Mass still present. Lesion stable but histologically positive.
10	Perianal adenoma	A	2	Tumor completely regressed but recurred after 4 months
11	Basal cell carcinoma	A	3	No evidence of neoplasma
12	Mast cell tumor	C,D	3	Some regression but still present, only partial response
13	Mast cell tumor	C,D	2	Mass continued to grow

*No physical evidence of tumor.
**Tumor regressed.

EXAMPLE 42

Canine patients were treated with Compounds A, C, D or E according to the protocol of Example 41. The results are given in Table 42.

TABLE 42

Lesion	Compound A Treatments	Cure	Partial	No Effect
Mast (3)	2-3	1	1	1
Squamous Cell (1)	3	1	-	-
Mammary (2)	2-3	1	-	1
Perianal (7)	2-3	1	1	5
Basal Cell (1)	3	1	-	-
Perianal Adenitis (1)	2	-	-	1
Perianal Cyst (benign) (1)	2	1	-	-

Lesion	Compound C Treatments	Compound D Treatments	Cure	Partial	No Effect
Mast Cell (7)	2-3	2-3	-	2	4

Lesion	Compound E Treatments	Cure	Partial	No Effect
Mast Cell	3	1	-	1

EXAMPLE 43

Equine patients with various tumor lesions were treated with Compound A, C or D. Melanoma, sarcoid and squamous cell carcinoma lesions were removed to skin level by surgical debulking; for papillomas, the lesion tips were removed. After hemostasis, the tumor site was covered liberally with the test compound extending 5 mm peripherially. Two weeks later, the crust was removed, the lesion area abraded and the test compound applied topically. After an additional two weeks, any crust was again removed from the lesion and the area abraded. The same test compound was again applied topically. Four weeks later, a biopsy of the lesion area was performed. The results of the equine studies are shown in Tables 43A and 43B.

TABLE 43A

Equine Studies Using Compound A

Equine Patient No.	Lesion/Location	Number Treatments	Final Biopsy Report
1	Papillomas on penis	2	Stratified squamous epithelium, acanthotic
2	Melanoma under tail	2	Acanthomatous epithelium and granulation tissue
3	Multiple papilloma on muzzle	3	No histologic lesion observed
4	Sarcoid on ear	3	Granulation tissue
5	Squamous cell carcinoma on prepuce	3	No evidence of recurrent tumor
6	Multiple papilloma on muzzle	3	No evidence of neoplasia
7	Squamous cell carcinoma on vulva	3**	Squamous cell carcinoma--low grade
8	Sarcoid on leg	3	Normal tissue
9	Flat sarcoid	2	Sarcoid regressive
10	Melanoma on vulva	2	Normal tissue
11	Squamous cell carcinoma on penis	3	No evidence of neoplasm

** Growth was greatly reduced and enabled delivery of normal foal which would not have been possible without treatment. Animal developed mastitis and purpura hemorrhagica, unrelated to treatment.

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TABLE 43A (Cont.)

Equine Studies Using Compound A

Equine Patent No.	Lesion/Location	Number Treatments	Final Biopsy Report
12	Sarcoid on shoulder	4*	No evidence of sarcoid
13	Sarcoid on ear	3	Scar tissue
14	Melanoma	3	Cure
15	Melanoma	3	Not effective
16	Papilloma	3	Cure

* Fourth treatment by accident.

TABLE 43B

Equine Studies with Compounds C and D

Equine Pateint No.	Lesion/ Location	Compound	Number Treatments	Final Biopsy Report
17	Sarcoid	C	3	No response
18	Sarcoid	D	3	Apparent elmination of tumor. Biopsy later.
19	Sarcoid	D	3	No response
20	Sarcoid	C	3	No response
21	Sarcoid	D	3	Some response
22	Sarcoid	C	3	Response unsatisfactory

EXAMPLE 44

Equine patients were treated with Compounds A, C, D or E according to the protocol of Example 43. The results are given in Table 44.

TABLE 44

Lesion	Compound A Treatments	Cure	Partial	No Effect
Papillomas (4)	2-3	4	-	-
Melanoma (4)	2-3	3	-	1
Squamous Cell Car. (3)	3	2	1	-
Sarcoid (5)	3	4	1	-

Lesion	Compound C Treatments	Compound D Treatments	Cure	Partial	No Effect
Sarcoid (6)	3	3	1	2	3

Lesion	Compound E Treatments	Cure		
Sarcoid (5)	3	5	-	-

EXAMPLE 45

This study was designed to provide basic pharmacokinetic data on the disposition of Carbon 14 (^{14}C) of nordihydroguaiaretic acid (NDGA) given dermally in modified compounds A and D. In addition, the distribution of zinc was measured for the dermally applied Compound A.

The ^{14}C-NDGA compound exhibited a specific radioactivity of 20.2 Ci/mol (66.9 micro Ci/mg) and a purity of 96.9% by mass spectrometry and by radioautography of thin-layer chromatography plates developed in benzene: isopropanol:acetic acid:water (25:5:2:10).

Subsequently, 25.1 mg of the ^{14}C-NDGA-Compound (66.9 micro Ci/mg) were mixed with 12.35 g of Compound A. Analyses of triplicate samples of the final mixture for ^{14}C by counting and for NDGA by high-pressure liquid chromatography (HPLC) demonstrated homogeneity, with a

content of 51 micro g of ^{14}C-NDGA compound/mg of Compound A. The specific radioactivity of the NDGA was 3.00 x 10^{3} micro Ci/micro g.

Similarly, 26.3 mg of the original ^{14}C-NDGA compound (66.9 micro Ci/mg) were mixed with 12.55 g of Compound A devoid of Z and EDTA to obtain a mixture for the study of the dermal penetration of NDGA from Compound A devoid of Z and EA. Analyses of triplicate samples of the modified Compound A showed the final mixture to be homogeneous with regard to ^{14}C and NDGA; it contained 53 micro g of ^{14}C-NDGA compound/mg of vehicle. The specific radioactivity of the diluted NDGA was 3.41 x 10^{-3} micro Ci/micro g.

The compounds were dermally administered to young adult Sprague-Dawley rats by the following protocol: Under ether anesthesia, the back skin of the rat was prepared by removing the hair from a 5 x 5-cm area with a clipper and the residual hair stubble was removed with a wax expilatory. Then the skin was stripped repeatedly (5x) with adhesive tape until the stratum corneum was removed. Then 0.5 gm of the formulation was weighed on a 5 x 5-cm sheet of polypropylene, which was applied to the prepared skin. It was secured in place by hypoallergenic tape. Finally, the bandage was overwrapped with bandage tape. After treatment, the rats were caged individually in metabolism cages, which allowed free access to food and water and provided for separate collection of urine and feces.

The testing of Compound A with ^{14}C-NDGA was performed in 15 male Sprague-Dawley rats (mean weight 339 ± 16 g). They received an average of 0.520 (± 0.032) g of Compound A containing ^{14}C-NDGA. The mean dose of ^{14}C-NDGA was 78.5 (± 7.0) mg/kg of body weight. The rats were housed individually in metabolism cages providing for free access to food and water and for the separate collection of urine and feces. Groups of 3 rats were

sacrificed at 4, 24, 48, 72, and 96 hours and excreta were collected from each rat during 24-hr periods. In addition to the usual collection of tissues, the skin site of application was excised after wiping the site with water-moistened tissue. The wipes were added to the wrappings, which were immersed in a small container of acetone.

The testing of Compound A devoid of Zn and EDTA was performed on 15 male Sprague-Dawley rats (mean weight 241 ± 7 g). They received an average of 0.390 (± 0.019) g of Zn-free C205 containing ^{14}C-NDGA. The average dose of ^{14}C-NDGA was 83.2 mg/kg of body weight. Groups of three rats were bled terminally and tissues were taken at 4, 24, 48, 72, and 96 hours after dosing. At each sacrifice time, those three rats scheduled to be sacrified next were also bled nonterminally from the orbital sinus. The wrappings and wipes of the skin site were taken at the time of sacrifice and added to acetone as described above.

The results of the study are given in Table 45A for Compound A containing ^{14}C-NDGA and Table 45B for Zn-Free Compound A containing ^{14}C-NDGA. The results of analysis for tissue distribution of zinc as a percent of dose in rats receiving Compound A containing ^{14}C-NDGA are given in Table 45C.

TABLE 45A

Tissue Distribution of ^{14}C as a Percent of Dose in Rats Receiving Compound A Containing ^{14}C-NDGA, Dermally

Tissue	Mean (± S.D.)[a] Percent of the Dose of ^{14}C Found at Hours				
	4	24	48	72	96
Organs (%)	4.62 (±2.27)	7.55 (±1.85)	10.29 (±8.8)	10.43 (±6.94)	13.45 (±3.45)
Skinsite (%)	20.8 (±8.4)	19.6 (±5.2)	11.1 (±1.4)	8.58 (±5.41)	7.01 (±2.28)

[a] N = 3

TABLE 45B

Tissue Distribution of ^{14}C as a Percent of Dose in Rats Receiving Zn-Free Compound A Containing ^{14}C-NDGA, Dermally

Tissue	Mean (± S.D.)[a] Percent of the Dose of ^{14}C Found at Hours				
	4	24	48	72	96
Organs (%)	10.28 (±6.12)	11.08 (±9.65)	10.47 (±10.33)	5.41 (±4.21)	8.03 (±1.48)
Skinsite (%)	4.15 (±0.76)	12.20 (±5.9)	7.86 (±3.75)	4.72 (±3.18)	1.43 (±0.55)

[a]N = 3

TABLE 45C

Tissue Distribution of Zn as a Percent of Dose in Rats Receiving Compound A, Dermally

Tissue	Mean Percent of the Dose of Zn found at Hours				
	4	24	48	72	96
Organs (%)	3.28	6.54	6.79	11.47	12.69
Skinsite (%)	10.5	11.8	9.58	6.46	4.99

[a]N = 3

The study was continued for testing Compound D with ^{14}C-NDGA; Zn- and EDTA-free Compound D; and modified Compound D with no BHT and 0.10 EDTA. The following Table 45D lists the compositions of the compounds and the amounts of materials used for preparing the compounds containing ^{14}C-NDGA. These compounds were analyzed for ^{14}C by scintillation counting and for NDGA by liquid chromatography.

TABLE 45D

Constituent	Compound D Formulation	Composition (%) Zn-Free Compound D Formulation	Modified Compound D Formulation
Compound Zn	10.00	0.00	10.00
Compound EDTA	4.93	0.00	0.10
Compound NDGA	4.60	4.60	4.60
Compound BHT	1.10	1.10	0.00
Water, purified	18.32	18.32	19.42
PEG 400	2.60	14.50	14.19
PEG 8000	53.45	49.39	46.69
Stearyl Alcohol	5.00	12.09	5.00
	100%	100%	100%
Compounds containing ^{14}C-NDGA:			
mg of ^{14}C-NDGA	25.75	25.40	25.20
g of Formulation	12.55	12.55	12.75
% NDGA in final mixture	4.80	4.79	4.78

The mean rat body weights, average doses of the formulations, and mean doses of ^{14}C-NDGA in mg/kg of body weight for the three current protocols were: 297 ± 15 g (standard deviation), 512 ± 28 mg, and 82.7± 2.0 mg/kg for Compound D; 325 ± 12 g, 570 ± 26 mg, and 84.0 ± 1.4 mg/kg for Zn-free Compound D; and 328 ± 27 g, 575 ± 45 mg, and 84.2 ± 2.8 mg/kg for modified Compound D. Fifteen rats were used for each study and groups of three rats were sacrificed at 4, 24, 48, 48, 72 and 96 hr after dosing. At each time, blood, liver, skin site, intestines and contents, carcass, and combined wrappings and wipes were collected. Also, from the groups sacrified at 24, 48, 72, and 96 hr urine, feces, and cage washings were collected.

The results of the study are given in Table 45E for Compound D; Table 45F for Zn-free Compound D; and Table 45G for modified Compound D.

TABLE 45E

Tissue Distribution of ^{14}C as a Percent of Dose in Rats Receiving Compound D Containing ^{14}C-NDGA, Dermally

Tissue	Mean (± S.D.)[a] Percent of the Dose of ^{14}C Found at Hours				
	4	24	48	72	96
Organs (%)	1.92 (±1.34)	4.41 (±1.44)	4.31 (±2.5)	3.38 (±0.52)	1.87 (±1.1)
Skinsite (%)	16.3 (±9.2)	11.9 (±6.1)	8.92 (±4.10)	6.55 (±1.76)	5.11 (±3.01)

[a]N = 3

TABLE 45F

Tissue Distribution of ^{14}C as a Percent of Dose in Rats Receiving Zn-Free Compound D Containing ^{14}C-NDGA, Dermally

Tissue	Mean (± S.D.)[a] Percent of the Dose of ^{14}C Found at Hours				
	4	24	48	72	96
Organs (%)	1.16 (±0.92)	2.29 (±2.26)	2.38 (±1.73)	7.39 (±10.05)	7.73 (±10.9)
Skinsite (%)	2.21 (±1.44)	4.45 (±4.36)	6.07 (±2.90)	18.6 (±6.3)	12.0 (±4.99)

[a]N = 3

TABLE 45G

Tissue Distribution of ^{14}C as a Percent of Dose in Rats Receiving Modified Compound D Containing ^{14}C-NDGA, Dermally

Tissue	Mean (± S.D.)[a] Percent of the Dose of ^{14}C Found at Hours				
	4	24	48	72	96
Organs (%)	4.35 (±3.06)	1.65 (±0.68)	4.41 (±3.57)	2.54 (±2.05)	0.97 (±0.19)
Skinsite (%)	14.4 (±7.1)	23.0 (±5.5)	17.1 (±4.7)	16.3 (±0.6)	17.7 (±6.2)

[a]N = 3

EXAMPLE 46

Cultures of representative microorganisms which included Gram negative and Gram positive bacteria, yeasts and molds were prepared to assess the effect of composition A of Example 36, as well as its separate components, on the survival and/or growth of the microorganisms. The microorganisms and the culture media used are given below.

- o Streptococcus sp., Group C, ATCC 9342 (Stp. Pyogenes, Lancefield Group A).
- o Staphylococcus aureus (penicillin sensitive), ATCC 9144
- o Staphylococcus aureus (penicillin resistant), ATCC 13301
- o Escerichia coli, ATCC 11229
- o Proteus mirabilis, ATCC 4675
- o Mycobacterium smegmatis, ATCC 20
- o Bacteroides fragilis, ATCC 23745
- o Candida albicans, ATCC 28366
- o Candida kruseri, ATCC 2159
- o Trichophyton mentagrophytes, ATCC 9533
- o Microsporum canis, ATCC 9084

All of the bacterial species, including M. smegmatis, were found to grow well in tryptic soy broth with dextrose (TSB). Good growth was also obtained with the yeast species in this medium. Although the fungal species grew in TSB, they grew somewhat better in Sabouruad's broth (SAB), and for the broth dilution tests with T. mentagrophytes and M. canis Sabouruad's was used. For spore production the fungal species were grown on malt-soil extract agar.

A series of tests were devised to determine the effect of direct exposure of the microorganisms to the test compositions. The tests were conducted according to the following general procedure.

Sterile tubes of a growth medium (broth) appropriate for the bacteria, yeast, or mold under test were inoculated and allowed to grow until the tube exhibited the maximum turbidity that could be expected for the particular species. For most bacteria and yeasts this time was 24 hours. For molds the procedure was different in that the fungal species were inoculated onto the surface of a malt extract-soil extract agar slant and allowed to grow at room temperature until a heavy mycelial growth with heavy spore production was observed. At this time the spores were washed from the mycelia with sterile water and agitation using a vortex mixer. The spore suspension was filtered through four layers of sterile cheesecloth into sterile tubes. The spore suspensions were handled from this point in the same manner as broth suspensions of bacteria or yeasts.

One milliliter of the bacterial, yeast, or mold spore suspension was transferred to a sterile 12-ml glass, conical, centrifuge tube covered with a sterile cap and centrifuged at 3,000 rpm for 15 min. Centrifugation was done at room temperature using a benchtop, angle-head, clinical centrifuge (Clay-Adams). After the bacteria, yeast, or mold spores were pelleted, the supernatant fluid was decanted and the tubes inverted over paper saturated with a biocide placed in a bacteriological hood.

The pellets in the centrifuge tubes were then mixed with 1 gram of the undiluted test material and allowed to remain in contact for 2 hours at 37°C for the bacteria and yeasts and at 25°C for the mold spores. At the end of the contact time, the test mixture was diluted 1 to 10 with growth medium (TSB or SAB broth). Additional serial

dilutions were made from the initial dilution up to 1 x 10^{-9}. Each material was tested in triplicate. The controls, which consisted of the microbial cells incubated with 1 gram of mineral oil, were diluted in the same way. All dilutions of both test materials and controls were then incubated at an appropriate temperature of 37°C for bacteria and yeasts and 25°C for molds to allow for growth of any viable cells present.

All bacterial species except M. smegmatis were incubated for 48 hours; M. smegmatis was incubated for 7 days. Yeast tests were incubated 48 hours. Molds were incubated for 10 days. For a determination of growth response, growth in tubes containing test compositions was compared to the growth in a mineral oil control at an equivalent dilution. Growth was indicated by turbidity in the broth medium.

Results of the direct exposure tests are given in Table 46.

TABLE 46

Direct Exposure Tests
(Organism Exposed to the Composition
for 2 Hours Prior to Dilution in Test Broth)

	Test Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	pH of Test Broth		5.5	6.8	7.0	7.0	7.0	7.0	7.0	7.0
	Precipitation of Test Compound	heavy ppt	heavy ppt	ppt	slight ppt	-	-	-	0	-
Streptococcus pyogenes	Growth in Test Broth	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control	+	+	+	+	+	+	+	+	+
Staphylococcus aureus (penicillin sensitive)	Growth in Test Broth	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control	+	+	+	+	+	+	+	+	+
Escherichia coli	Growth in Test Broth	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control	+	+	+	+	+	+	+	+	+

1. No growth shown in any of the three tubes at that dilution.
2. Indicates growth of cells occurred.

TABLE 46 (Cont.)

	Test Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	pH of Test Broth		5.5	6.8	7.0	7.0	7.0	7.0	7.0	7.0
	Precipitation of Test Compound	heavy ppt	heavy ppt	ppt	slight ppt	-	-	-	0	-
Mycobacterium smegmatis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Control	-	+	+	+	0	0	0	0	0
Bacteriodes fragilis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Control	-	+	+	+	+	0	0	0	0
Candida albicans	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0
	Growth in Mineral Oil Conrol	-	+	+	+	+	+	+	+	0
Microsporum canis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Conrol	-	+	+	+	+	0	0	0	0

EXAMPLE 47

Tests were conducted on the effect of the direct exposure of representative microorganisms to several separate components of the composition of Example 36. Pego base alone was tested in one series of evaluations to determine whether or not inhibition by this carrier would have to be considered in evaluating the results of the individual ingredients dissolved in it.

In order to better approximate the effects of pego base in the Example 36 formulations, the amount of polyethylene glycol present in the formulation was calculated. The pure base material was then diluted with water to this concentration. Mineral oil was used as a positive control.

Nordihydroguaiaretic acid (NDGA) and desmethyl NDGA (DM-NDGA) diluted in pego base were also tested for inhibitory properties against representative gram-negative and gram-positive bacteria and yeasts covering the spectrum of microorganisms used in these tests.

The initial concentration of the compounds tested was equivalent to the amount present in the composition, and the general procedure outlined in Example 46 was followed. After a 2-hour exposure of the microorganisms to this initial concentration, progressive 1 to 10 serial dilutions of the mixture were made to assess viability of any microorganisms present. Results are shown in Table 47.

TABLE 47

Growth of Selected Microorganisms
Following Direct Exposure Tests
to NDGA, Desmethyl NDGA, PEGO

Microorganism Tested	NDGA[1] micrograms/ml				DM-NDGA[1] micrograms/ml				PEGO[1] micrograms/ml				Mineral Oil micrograms/ml			
Concentration of Test Material	4,600	460	46	4.6	1,100	110	11	1.1	310	31	3.1	0.31	500	50	5.0	0.5
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Streptococcus pyogenes	0	0	0	0	0	0	0	0	2^+	2^+	2^+	2^+	4^+	4^+	4^+	4^+
Escherichia coli	2^+ [2]	4^+ [3]	4^+ [4]	4^+	0	0	4^+	0	4^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+
Staphylococcus aureus (Penicillin Resistant)	0	0	4^+	4^+	0	0	4^+	4^+	2^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+
Candida albicans	0	0	2^+	2^+	±	1^+	3^+	3^+	3^+	3^+	3^+	3^+	4^+	4^+	4^+	4^+

1/ All test media at all dilutions were at pH 7.3.
2/ A heavy precipitate formed and bacterial turbidity was estimated; there was obvious growth with heavy production of gas.
3/ Moderate precipitate.
4/ Light precipitate.

EXAMPLE 48

A combination of EDTA (ethylenediaminetetraacetic acid) and zinc chloride in pego base at the concentration in which these components are present in composition A of Example 36 was tested for its effect on the viability of representative microorganisms. All organisms given in Example 46 were tested except Candida kruseri, and Microsporum canis.

The test procedure followed was that generally described in Example 46. The initial test composition of EDTA/zinc to which the microorganisms were exposed for 2 hours of direct contact had a pH of about 2.0. None of the organisms tested retained viability after exposure to this test mixture. All mineral oil controls showed abundant (4^+) growth.

In every test a heavy precipitate formed when the test mixture was diluted to 10^{-2}. The pH of the 10^{-2} dilution was 4.75. No precipitate formed at the 10^{-1} dilution (pH 1.8), the 10^{-3} dilution (pH 6.60) or the 10^{-4} dilution (pH 7.0).

EXAMPLE 49

A test was conducted to assess the growth of Escherichia coli and Staphylococcus aureus in broth containing composition A of Example 36, NDGA or desmethyl NDGA diluted in glycerol. Test parameters and results are given below.

TABLE 49

	Growth (48 hr at 37°C)	
	E. coli	S. aureus
Glycerol		
1 ml in 10 ml TSB	4+	4+
0.1 ml in 10 ml TSB	4+	4+
NDGA in Glycerol		
100 ppm (as NDGA) in TSB	3+	0
1,000 ppm (as NDGA) in TSB	3+	0
Desmethyl NDGA in Glycerol		
100 ppm (as DM-NDGA) in TSB	3+	0
1,000 ppm (as DM-NDGA) in TSB	3+	0
Compound A in Glycerol		
100 ppm	4+	4+ (4.6 ppm NDGA + 1.1 ppm BHT)
1,000 ppm	4+	0 (46 ppm NDGA + 11 ppm BHT)

EXAMPLE 50

A series of broth dilution tests were conducted to assess the effect of composition A of Example 36 and its separate components on the growth of microorganisms. The individual test materials were incorporated into pego base at the concentration in which they are present in the composition for testing. EDTA and zinc chloride were tested together. Each original formulation was diluted 1 to 10 with growth medium, (usually tryptic soy broth with glucose), and subsequent 1 to 10 dilutions were made of the previous dilution usually up to 1×10^{-4}. This test was done with no consideration given to the solubility of the test material when diluted. In all cases, controls consisting of cells in mineral oil diluted in TSB were made to test the effect of the medium on growth. The pH determination of each series of materials was made by testing a duplicate set of tubes that were uninoculated. Each dilution tube containing 10 ml. test broth was inoculated with 0.1 ml. of a 24-hour culture of all test species except M. smegmatis and the mold species. Spore suspensions of fungi (10 days) were used to inoculate the tubes for testing effects on M. canis and T. mentagrophytes, and Sabouraud's broth was used for dilution because the fungal species grew somewhat better in this medium than in TSB. In general, a stationary phase culture of each test species was used.

Results of the broth dilution tests are given in Table 50. The pH values given in the tables apply only to the dilution shown.

The readings of turbidity in the growth media which indicate growth of the microorganism are rated from 0 = no growth, to 4+ = turbidity equal to the control. A 4+ reading for one microbial culture does not mean that the turbidity of that culture was the same as a 4+ reading

for any other culture. A 4+ reading means that turbidity in the tubes of a particular test was equal to the turbidity of the appropriate control at the dilution compared.

TABLE 50

Broth Dilution Tests

Test Microorganism	Test Compound	Compound A				Control Pego Base			
	Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	Broth pH	2.6	5.2	6.6	6.6	7.1	7.1	7.1	7.1
Streptococcus pyogenes		0	0	0	4^+	4^+	4^+	4^+	4^+
Staphylococcus aureus (Pen. Resistant)		0	0	0	4^+	4^+	4^+	4^+	4^+
Staphylococcus pyogenes (Pen. Sensitive)		0	0	0	4^+	1^+	4^+	4^+	4^+
Escherichia coli		0	0	4^+	4^+	4^+	4^+	4^+	4^+
Proteus mirabilis		0	0	4^+	4^+	4^+	4^+	4^+	4^+
Mycobacterium smegmatis		0	0	0	2^+	±	4^+	4^+	4^+
Bacteriades fragilis		0	0	0	0	4^+	4^+	4^+	4^+
Candida albicans		0	1^+	4^+	4^+	2^+	4^+	4^+	4^+
Candida kruseri		0	0	2^+	3^+	4^+	4^+	4^+	4^+
Trichophyton mentagrophytes		0	0	2^+	2^+	4^+	4^+	4^+	4^+
Microsporum canis		0	0	4^+	4^+	not tested			

EXAMPLE 51

Compositions containing NDGA, zinc chloride or a combination of NDGA-Zn were tested for antimicrobial activity individually against seven gram positive and gram negative bacterias, yeasts and molds.

In a preliminary screening test, a vehicle containing 30% polyethylene glycol-200 (PEGO-200) + 0.1% sodium ascorbate in deionized water at various concentrations was shown to exhibit no inhibitory effect on microbial growth and was chosen as the diluent for the test compounds. Stock solutions of the test compounds in 30% PEGO-200/water were prepared at the following weight percent concentrations: 4.6% NDGA + 0.1% ascorbic acid; 5.0% $ZnCl_2$ + 0.1% sodium ascorbate; and 4.6% NDGA + 5.0% $ZnCl_2$ + 0.1% sodium ascorbate. Aliquots of the stock solutions were diluted 1:10 and 1:100 with the 30% PEGO-200 diluent. The stock solutions were further diluted 1:10 with Brain Heart Infusion Agar, which was melted at 45°C prior to the addition of the test solutions. The agar containing the test solutions was then poured into 50 x 90 mm petri dishes and allowed to dry for four hours at room temperature prior to inoculation.

All Brain Heart Infusion slants were started at 35°C anaerobically except for T. mentagrophytes at 27°C and P. acnes at 35°C anaerobically. Those microbial slants incubated at 35°C were subsequently transferred to new slants at 35°C and incubated at the same temperature. All slants were harvested with 1 ml saline containing 0.05% Tween-80 and diluted with saline in the following amounts to be used as working inocula: 1 ml each of E. Coli, P. aeruginosa, S. aureus and B. subtilis was diluted with 99 ml saline; 1 ml of C. albicans and P. acnes was diluted with 9 ml saline; 1 ml of T. mentagrophytes was left undiluted.

One drop of working inocula was added to the petri dishes containing the test compounds and allowed to

absorb into the agar. Uninoculated (control) and inoculated dishes were sealed and incubated in the dark for 5-7 days under the following conditions: P. acnes anaerobically at 35°C, T. mentagrophytes at 27°C and the remaining at 35°C aerobically. The plates were visually observed for microbial growth. Table 51A shows the dose levels and inhibitory effects of the test compounds. Table 51B provides a summary of the results with the test compounds showing the lowest dosage with complete inhibition.

TABLE 51A

% Active(s) in Agar	E. coli	P. aeruginosa	S. aureus	B. subtilis	P. acnes	C. albicans	T. mentogrophytes
0.46% NDGA	-	-	++	++	++	++	+
0.046% NDGA	-	-	++	++	++	+	-
0.0046% NDGA	-	-	-	-	-	-	-
0.5% $ZnCl_2$	++	++	++	++	++	++	++
0.05% $ZnCl_2$	-	-	-	+	+	-	-
0.005% $ZnCl_2$	-	-	-	-	-	-	-
0.46% NDGA + 0.5% $ZnCl_2$	++	++	++	++	++	++	++
0.046% NDGA + 0.05% $ZnCl_2$	-	-	++	++	++	+	+
0.0046% NDGA + 0.005% $ZnCl_2$	-	-	-	-	++	-	-
Vehicle Control (with 0.1% Sodium Ascorbate)							
0.2% Polyethylene Glycol-200	-	-	-	-	-	-	-
2.0% Polyethylene Glycol-200	-	-	-	-	-	-	-
20.0% Polyethylene Glycol-200	-	-	-	-	-	-	-

Results are found in triplicate samples:

++ = Complete inhibition of growth

+ = Partial inhibition (some growth)

- = Little or no inhibition (good growth)

TABLE 51B

Test Compound	E. coli	P. aeruginosa	S. aureus	B. subtilis	P. acnes	C. albicans	T. mentogrophytes
NDGA Alone	ND	ND	0.046%	0.046%	0.046%	0.46%	ND
$ZnCl_2$ Alone	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Combination							
NDGA +	0.46%	0.46%	0.046%	0.046%	0.0046%	0.46%	0.46%
$ZnCl_2$	0.5%	0.5%	0.05%	0.05%	0.005%	0.5%	0.5%

ND = Complete inhibition of growth was not detected

EXAMPLE 52

The in vitro antitumor effect of the interaction of NDGA and $ZnCl_2$ at various ratios were determined utilizing the clonogenetic assay. The human lung tumor cell line, LX-T, which was derived from the solid tumor, LX-1, was cultured in the standard medium RPMI-1640 + 10% fetal calf serum (FCS). A stock solution (10^{-2}M) of NDGA was prepared by dissolving 32.04 mg of NDGA in 4 ml DMSO and 6 ml distilled H_2O. A 10^{-2}M stock solution of $ZnCl_2$ was prepared by dissolving 13.63 mg $ZnCl_2$ in 10 ml of distilled H_2O. Serial dilutions of the test stock solutions (ranging from 25-200 microliters) were made in 15 ml of Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS). Harvested LX-T cells with greater than 90% viability were added to 8 ml of each test solution in HBSS and incubated for 1 hour at 37°C. The cells were then washed two times with HBSS and resuspended with 12 ml of plating medium prepared as follows: 25 ml of 2x RPMI-1640 + 25 ml of 3% Agarose in H_2O + 7.5 ml FCS to provide a final concentration of 3.3×10^4 cells/ml. After each washing the cells were aseptically centrifuged and the supernatant decanted from the pellet. Two ml of the plating media cell mixture were then added to each gridded 100 mm diameter plate, and the plates were placed in the refrigerator for 10 minutes. The remaining plating media cell mixtures contained in the tubes were placed on ice for 10 minutes. The plates and tubes were then incubated at 37°C for three days. The tubes were placed in a water bath at 56°C for 10 minutes and warm HBSS was added to wash out the agarose. The cells and colonies were washed two times with HBSS. The pellet was resuspended in 1 ml of HBSS and analyzed by flow cytometry forward and right angle light scatter.

Visual Colony Counting: Two ml of the 1.0% agarose with the cells were poured into a 100 mm diameter gridded

plate (Falcon). The plates were placed in the refrigerator for 10 min., and then incubated at 37°C for 3 days. Each sample was prepared in triplicate and a minimum of 200 cells/colonies were counted. These values were averaged to determine the % colonies formed.

Flow Cytometry Colony Counting: Freshly prepared single cell suspensions of LX-T cells were analyzed by foward and right angle light scatter using the Ortho System 50 Cytofluorograf with the coherent 5-watt Argon ion Laser and 2150 computer system. An integration boundary was formed around the single and double cell light scatter distribution. A second boundary was established which included the single cells and colonies. These boundaries excluded any small particles which were carried over from the agarose washings (1-2 micrometers, um). The diameter range for the LX-T cells was 15-20 um, while the colonies formed after 3 days incubation were 50-100 um.

DNA Flow Cytometric Analysis: After LX-T cells were incubated in the presence of the drugs, they were harvested by slight scraping with a rubber policeman ad centrifuged at 500 xg, 100 min., at room temperature. The cells and colonies were resuspended in a solution of the DNA-specific fluorochrome, (4', 6-Diamidino - 2 - phenylindole (DAPI) (polysciences) dissolved in a speical nuclear isolation medium. The suspension of nuclei was passed through a 70 um filter and stored on ice. Trout red blood cells (TRBC's) were used as the DNA standard (5.0 pg/nucleus) for these studies as described previously to quantitate the DNA content of the LX-T nuclei. The DNA distributions of the DAPI-stained nuclei were obtained as previously described.

The results from these evaluations are provided in the following tables. Tables 52A, 52B and 52C contain results using ratios of NDGA:$ZnCl_2$ of 1:0.5, 1:2 and 1:5

respectively. Tables 52D and 52E contain results using NDGA alone and $ZnCl_2$ alone respectively.

Using the data in Tables 52A-E, the effective doses at different responses (EDx) were calculated. These values are provided along with the +-standard deviation of each. The results for NDGA alone and for NDGA in combination with $ZnCl_2$ are given in Table 52F. The calculated effective doses for $ZnCl_2$ alone and for $ZnCl_2$ in combination with NDGA are given in Table 52G. All values are in micromoles.

Table 52H contains results of evaluations of three known anticancer drugs. The $ED_{(50)}$ for each was calculated using the data in Table 52H. These values along with $ED_{(50)}$ for NDGA are given in Table 52I.

TABLE 52A
NDGA:$ZnCl_2$ (1:0.5)

No.	NDGA Dose microM	NDGA Dose Ln	$ZnCl_2$ Dose microM	$ZnCl_2$ Dose Ln	% Response
A	6.00	1.79	3.00	1.10	0.7
	8.00	2.08	4.00	1.39	5.0
	10.00	2.30	5.00	1.61	18.3
	12.00	2.48	6.00	1.79	32.2
	15.00	2.71	7.50	2.01	34.4
	18.00	2.89	9.00	2.20	41.6
B	8.0	2.08	4.0	1.39	22.2
	10.0	2.30	5.0	1.61	27.2
	15.0	2.71	7.5	2.01	65.7
	18.0	2.89	9.0	2.20	77.1
	21.0	3.04	10.5	2.35	81.4
	24.0	3.18	12.0	2.48	82.6
C	8.0	2.08	4.0	1.39	17.9
	10.0	2.30	5.0	1.61	37.1
	15.0	2.71	7.5	2.01	83.4
	18.0	2.89	9.0	2.20	87.5
	21.0	3.04	10.5	2.35	88.3
	24.0	3.18	12.0	2.48	88.4
D	5.00	1.61	2.50	0.92	18.4
	10.00	2.30	5.00	1.61	28.9
	15.00	2.71	7.50	2.01	65.2
	20.00	3.00	10.00	2.30	80.7
	25.00	3.22	12.50	2.53	90.4

TABLE 52B
NDGA:$ZnCl_2$ (1:2)

No.	NDGA Dose microM	NDGA Dose Ln	$ZnCl_2$ Dose microM	$ZnCl_2$ Dose Ln	% Response
E	2.8	1.03	5.6	1.72	5.7
	3.0	1.10	6.0	1.79	10.7
	3.5	1.25	7.0	1.95	22.4
	4.0	1.39	8.0	2.08	36.1
	4.5	1.50	9.0	2.20	39.7
	5.0	1.61	10.0	2.30	49.6
F	2.8	1.03	5.6	1.72	34.5
	3.0	1.10	6.0	1.79	31.5
	3.5	1.25	7.0	1.95	47.6
	4.0	1.39	8.0	2.08	56.9
	4.5	1.50	9.0	2.20	69.8
	5.0	1.61	10.0	2.30	67.1
G	4.00	1.39	8.00	2.08	23.4
	5.00	1.61	10.00	2.30	33.4
	7.50	2.01	15.00	2.71	64.8
	10.00	2.30	20.00	3.00	71.5
	15.00	2.71	30.00	3.40	68.3
H	1.00	0.00	2.00	0.69	15.0
	2.50	0.92	5.00	1.61	29.2
	4.00	1.39	8.00	2.08	34.0
	5.00	1.61	10.00	2.30	64.7
	7.50	2.01	15.00	2.71	90.2

TABLE 52C

NDGA:$ZnCl_2$ (1:5)

No.	NDGA Dose microM	NDGA Dose Ln	$ZnCl_2$ Dose microM	$ZnCl_2$ Dose Ln	% Response
I	2.0	0.69	10.0	2.30	26.9
	4.0	1.39	20.0	3.00	34.3
	6.0	1.79	30.0	3.40	54.4
	8.0	2.08	40.0	3.69	64.6
	10.0	2.30	50.0	3.91	62.0
J	0.5	-0.69	2.5	0.92	16.0
	1.0	0.00	5.0	1.61	22.2
	1.5	0.41	7.5	2.01	22.7
	2.0	0.69	10.0	2.30	47.2
	2.5	0.92	12.5	2.53	58.1
	3.0	1.10	15.0	2.71	61.3
K	0.5	-0.69	2.5	0.92	4.0
	1.0	0.00	5.0	1.61	17.6
	1.5	0.41	7.5	2.01	18.0
	2.0	0.69	10.0	2.30	69.9
	2.5	0.92	12.5	2.53	72.3
	3.0	1.10	15.0	2.71	71.3
L	2.00	0.69	10.00	2.30	22.5
	3.00	1.10	15.00	2.71	59.5
	4.00	1.39	20.00	3.00	86.5
	5.00	1.61	25.00	3.22	87.8
	6.00	1.79	30.00	3.40	87.8

TABLE 52D
NDGA Alone

No.	Dose microM	Ln	Response (%)
1	11.85	2.47	29.5
	12.35	2.51	46.0
	14.81	2.70	14.0
	23.70	3.17	80.0
	23.70	3.17	76.5
	24.69	3.21	81.0
	37.04	3.61	95.0
	49.38	3.90	99.0
	74.07	4.31	99.8
2	10	2.30	18.6
	15	2.71	15.3
	20	3.00	45.6
	25	3.22	65.4
	30	3.40	60.2
3	16.00	2.77	52.5
	18.00	2.89	70.2
	22.00	3.09	84.7
	25.00	3.22	86.2

TABLE 52E
$ZnCl_2$ Alone

No.	Dose microM	Ln	Response (%)
4	3	1.10	13.8
	6	1.79	25.5
	9	2.20	31.6
	12	2.48	35.8
	18	2.89	63.3
	24	3.18	85.5
5	10	2.30	0.0
	10	2.30	2.8
	15	2.71	18.5
	15	2.71	23.5
	20	3.00	39.2
	25	3.22	47.9
	25	3.22	56.2
	30	3.40	54.7
	30	3.40	60.8
6	10.00	2.30	21.8
	15.00	2.71	60.0
	17.00	2.83	68.7
	20.00	3.00	75.9
	22.00	3.09	77.1

TABLE 52F

Calculated ED_x for NDGA

NDGA Average*		$ZnCl_2$:NDGA 0:1	$ZnCl_2$:NDGA 2:1	$ZnCl_2$:NDGA 0.5:1	$ZnCl_2$:NDGA 5:1
ED50	*	17.6 +- 4.07	4.7 +- 1.91	13.0 +- 3.08	3.1 +- 1.92
ED75	*	26.6 +- 12.11	7.9 +- 4.60	18.5 +- 3.07	7.0 +- 6.72
ED90	*	41.1 +- 28.10	13.6 +- 10.71	26.4 +- 3.82	16.7 +- 21.06
ED95	*	55.9 +- 46.79	20.1 +- 18.38	33.7 +- 5.75	31.2 +- 44.24

* All concentrations in microM + - Standard Deviation.

TABLE 52G

Calculated ED_x for $ZnCl_2$

$ZnCl_2$ Average*		$ZnCl_2$:NDGA 1:0	$ZnCl_2$:NDGA 2:1	$ZnCl_2$:NDGA 0.5:1	$ZnCl_2$:NDGA 5:1
ED50	*	21.3 +- 7.16	9.5 +- 3.81	6.5 +- 1.54	15.7 +- 9.60
ED75	*	32.9 +- 10.80	15.8 +- 9.21	9.2 +- 1.53	35.0 +- 33.60
ED90	*	51.7 +- 19.16	27.3 +- 21.43	13.2 +- 1.91	83.6 +- 105.30
ED95	*	71.0 +- 30.11	40.1 +- 36.77	16.9 +- 2.89	155.7 +- 221.22

* All concentrations in microM + - Standard Deviation.

TABLE 52H

Known Anticancer Agents

Adriamycin			5-Fluorouracil			Mutamycin		
Dose			Dose			Dose		
microM	Ln	% Response	microM	Ln	% Response	microM	Ln	% Response
9.20	2.2	1.9	19.22	3.0	0.1	2.30	0.8	0.0
9.20	2.2	4.2	25.37	3.2	7.0	2.30	0.8	0.0
9.20	2.2	7.2	76.86	4.3	6.5	2.30	0.8	0.1
9.20	2.2	39.8	102.23	4.6	27.8	4.61	1.5	0.0
18.40	2.9	40.4				4.61	1.5	0.0
18.40	2.9	41.0				4.61	1.5	0.4
18.40	2.9	43.4				9.22	2.2	0.0
18.40	2.9	46.7				9.22	2.2	0.0
18.40	2.9	46.7				9.22	2.2	4.4
27.60	3.3	48.7				13.82	2.6	26.6
27.60	3.3	54.8				13.82	2.6	28.6
36.80	3.6	71.1				18.43	2.9	37.1
36.80	3.6	73.2				18.43	2.9	40.1
36.80	3.6	75.9						
73.59	4.3	84.1						
73.59	4.3	84.4						
73.59	4.3	85.5						

TABLE 52I

Drug Name	ED(50) microM
5-FU	102.23
Adriamycin	25.6
Mutamycin	18.43
NDGA	17.6

EXAMPLE 53

The in vivo antitumor effect of the interaction of NDGA and $ZnCl_2$ at various ratios was determined against MX-1 (human breast adenocarcinoma) cells. Male or female athymic Balb/c mice, six to eight weeks of age and weighing 20 to 35 grams were used. MX-1 cells were cultured in the standard RPMI-1640 media and implanted subcutaneously in the flank of nude mice in order to propogate the tumor line. Nude mice were implanted with 25 mg of the MX-1 solid tumor fragments. Tumors which reached the 25-100 mm^2 range were used for the experiment. 0.1 ml of the test compound was injected directly into the tumor. The tumors were measured periodically to determine their weight calculated by using half the product of the length times the width times the height of the tumor. The procedure was repeated at regular intervals until 60 days after the initial treatment or all mice had died. Mice which showed no evidence of tumors were kept for 60 days to evaluate potential for tumor recurrence, at which time tumor characteristics, if any, were recorded. Table 53A contains the results of the runs using mixtures of NDGA and $ZnCl_2$. Table 53B and 53C respectively contain the results of runs with NDGA alone and $ZnCl_2$ alone.

Using the data in Tables 53A, 53B and 53C, the effective doses (ED_x) at different response levels were determined mathematically. These values for $ZnCl_2$ alone, and of $ZnCl_2$ in combination with NDGA are provided in Table 53E. The effective doses of NDGA alone and of NDGA in combination with $ZnCl_2$ are provided in Table 53E.

TABLE 53A

NDGA + $ZnCl_2$

NDGA:$ZnCl_2$ Ratio	Dose NDGA (micromoles)	Dose $ZnCl_2$ (micromoles)	Ln (NDGA + $ZnCl_2$)	Response (%)
1:1	3.14	2.20	1.68	10
	6.22	6.38	2.53	40
	9.13	9.61	2.93	78
1:2	1.55	2.79	1.47	33
	4.56	9.54	2.65	38
	7.47	16.29	3.17	67
1:5	0.99	4.33	1.67	0
	1.98	10.05	2.49	22
	3.84	21.94	3.25	80

TABLE 53B

NDGA Alone

Dose micromoles	%	Ln	% Response
5.16	1.56	1.64	20.0
7.04	2.13	-	0.0
8.30	2.51	2.12	10.0
10.42	3.15	-	0.0
11.58	3.50	2.45	30.0
13.96	4.22	2.64	40.0
15.97	4.83	2.77	44.4
17.53	5.30	2.86	40.0
21.10	6.38	3.05	62.5
29.24	8.84	3.38	88.9
40.38	12.21	3.70	70.0
58.51	17.69	4.07	70.0
81.69	24.70	-	62.5

TABLE 53C

$ZnCl_2$ Alone

Dose micromoles	%	Ln	% Response
5.36	0.73	1.68	0.0
7.34	1.00	1.99	0.0
10.71	1.46	2.37	38.5
12.84	1.75	2.55	50.0
13.58	1.85	2.61	33.3
16.88	2.30	2.83	50.0
17.83	2.43	2.88	55.6
21.13	2.88	3.05	55.6
26.20	3.57	3.27	90.0

TABLE 53D

ED_x (micromoles) for $ZnCl_2$ Alone and in Combination

$ZnCl_2$ Response Level	1:0 $ZnCl_2$:NDGA	2:1 $ZnCl_2$:NDGA	1:1 $ZnCl_2$:NDGA
ED(50)	15.65	4.57	5.70
ED(75)	22.23	6.21	8.78
ED(90)	31.58	8.44	13.55
ED(95)	40.10	10.41	18.19

TABLE 53E

ED_x (micromoles) for NDGA Alone and in Combination

NDGA Response Level	0:1 $ZnCl_2$:NDGA	2:1 $ZnCl_2$:NDGA	1:1 $ZnCl_2$:NDGA
ED(50)	13.62	2.28	5.70
ED(75)	25.66	3.10	8.78
ED(90)	48.33	4.22	13.55
ED(95)	74.34	5.20	18.19

EXAMPLE 54

Liquid samples were prepared containing 50 volume percent each of water and ethanol, and 10 weight percent NDGA and 2.5 weight percent of the indicated metal ion. The pH of the water was adjusted to either 4, 7 or 10, as indicated. The solutions were placed in quartz tubes in the cavity holder of a Bruker ER 200D electron spin resonance (ESR) spectrometer. Initiation was accomplished with the addition of 1 weight percent of ammonium persulfate and 0.05 weight percent N,N,N',N'-tetramethylenediamine (TMD) dissolved in ethanol. An ESR signal was commonly observed immediately after initiation with the TMD. The ESR signal was monitored over the indicated time periods. The maximum peak to minimum peak signal

height values were recorded and used as a relative measure of the radical concentrations and lifetimes. In the following tables, "t" is the time in minutes after initiation and the "relative peak height" is the distance between the maximum peak and minimum peak in arbitrary (but consistent) units recorded at time "t". Values are ±1 unit.

All reagents used were "Baker analyzed" obtained from the Fisher Scientific Company.

Relative ESR signal intensity measured at different time periods after initiation are given in Table 54A for NDGA at a pH of 4 in the presence and absence of zinc or magnesium metal ions. A duplicate run "B" was made for NDGA with $ZnCl_2$.

Relative ESR signal intensities are given in Table 54B at different time periods at a pH of 7 for NDGA alone, NDGA with zinc ions, and NDGA with magnesium ions.

Table 54C contains values for relative ESR signal intensity at different time periods after initiation at a solution pH of 10 for NDGA alone, NDGA with zinc ions, and NDGA with magnesium ions.

NDGA was separately tested with ferrous ions and cobalt (II) ions at a solution pH of 7. No ESR signal could be detected.

Dopa, 3-hydroxytyrosine, was used instead of NDGA both with and without zinc chloride at a pH of 7. No ESR signal was detected with dopa alone. When dopa was combined with zinc chloride at a pH of 7, the results in Table 54D were obtained.

Relative rate constants for the various compositions were determined using a standard equation,

$$Kd = \frac{\text{slope of curve}}{(\text{DOPA radical concentration})\ (60\ \text{sec/min})}$$

The values were normalized to the reported literature value for initial semiquinone free radical ion concen-

tration for dopa of 10^{-8}M. (B. Kalyanaranan et al., J. Biological Chem., Vol. 259, No. 12, pp. 754-89, 1984, which is incorporated herein by reference). The "slope of curve" is the rate of change of free radical concentration per unit of time determined from the data in the Tables in the usual way of 10^{-8}M. The various rate constants Kd are given in Table 54E. These results show that there is a substantial decrease in the rate of decay of the NDGA semiquinone radical/radical anion intermediate when zinc is present as opposed to magnesium, iron or cobalt. This indicates that zinc acts to stabilize the radical intermediate formed during oxidation which effectively stabilizes the NDGA.

TABLE 54A
pH 4

NDGA Alone		NDGA + $MgCl_2$	
t	relative peak height	t	relative peak height
0	12	0	17
5	9	5	10
10	7	10	8
15	6	15	6
20	5		

NDGA + $ZnCl_2$				
t	relative peak height		t	relative peak height
	A	B		A
0	69	71	80	24
5	67	59	90	23
10	64	55	100	22
15	57	52	110	21
20	50	50	120	20
25	47	-	130	19
30	44	-	140	20
35	40	-	150	17
40	37	-	160	16
50	33	-	170	15
60	29	-	175	15
70	26	-		

TABLE 54B
pH 7

NDGA			NDGA + $MgCl_2$		
t	relative peak height		t	relative peak height	
	A	B		A	B
0	9	9.4	0	13	16
5	7	6.7	5	10	10
10	5	5.2	10	8	7
15	4	4	15	-	6
			20	5	5
			25	-	4

NDGA + $ZnCl_2$		
t	relative peak height	
	A	B
0	71	65
5	43	47
10	27	30
15	21	21
20	14	15
30	-	11

TABLE 54C
pH 10

NDGA		NDGA + $MgCl_2$	
t	relative peak height	t	relative peak height
0	13	0	63
5	12	4	55
10	9	7	45
15	9	17	25
20	7	20	26
25	7.7	27	14
30	7	30	20
40	6		

NDGA + $ZnCl_2$

t	relative peak height
0	100
30	62.5
60	36
94	18
120	11
150	7
180	6

TABLE 54D
DOPA + $ZnCl_2$ @ pH 7

t	relative peak height
0	14.3
3	9
5	7

TABLE 54E

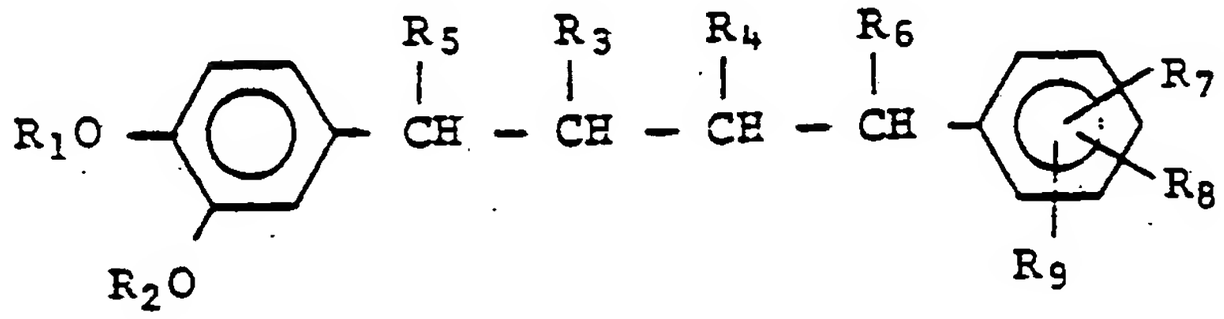
Rate Constants Kd

Solution Composition	pH	Kd(M^{-1} sec^{-1})
NDGA	4	$1.05x10^4$
	7	$1.05x10^4$
	10	$4x10^3$
NDGA + $MgCl_2$	4	$1x10^4$
	7	$1.3x10^4$
	10	$2.9x10^3$
NDGA + $ZnCl_2$	4	$5.1x10^2$
	7	$4.2x10^3$
	10	$1.7x10^2$
NDGA + $FeCl_2$	7	undetectable signal
NDGA + $CoCl_2$	7	"
DOPA	7	"
DOPA + $ZnCl_2$	7	$1-2x10^4$

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention, as limited only by the scope of the appended claims.

We Claim:

1. A pharmaceutical composition adapted for applying to an afflicted situs said composition comprising (a) a catecholic butane of formula:



wherein R_1 and R_2 are independently H, C_1-C_6 alkyl, or a C_7 or lower acyl;
R_3 and R_4 are independently H, CH_3, or C_2H_5;
R_5 and R_6 are independently H or OH; and
R_7, R_8 and R_9 may be attached at any separate position C_2-C_6 of the benzene ring and are independently H, OH, or OR_1 wherein R_1 is as defined above;
and a non-escharotic concentration of a pharmaceutically acceptable source of ionic zinc;
(b) a zinc salt or chelate of said catecholic butane; or
(c) a mixture of (a) and (b) wherein in (a), (b) and (c) the molar ratio of said catecholic butane to said ionic zinc is between about 5 to 1 and about 1 to 20.

2. The composition of Claim 1 wherein said catecholic butane is selected from the group consisting of 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dimethoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dibutoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-diacetoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dihydroxyphenyl)butane; 1-(3,4-dihydroxyphenyl)-4-(3,4,5-trihydroxyphenyl)butane; 1-(3,4-dihydroxyphenyl)-4-(3,4,5-trihydroxyphenyl)-2,3-dimethylbutane; and 1-(3,4-dihydroxyphenyl-4-(2,5-dihydroxyphenyl)butane.

3. The composition of Claim 1 wherein said molar ratio is between about 3 to 1 and about 1 to 10.

4. The composition of Claim 2 wherein said 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane is the meso-isomer, d-isomer, l-isomer, racemic d,l- mixture, or mixtures thereof.

5. The composition of Claim 1 wherein said source of ionic zinc comprises zinc chloride, bromide, iodide, nitrate, phosphate, sulfate, acetate, gluconate, benzoate, citrate, caprylate, or mixtures thereof.

6. The composition of Claim 5 wherein said source of ionic zinc comprises zinc chloride.

7. The composition of Claim 1 comprising a pharmaceutically acceptable carrier or adjuvant.

8. The composition of Claim 7 wherein said catecholic butane and said ionic zinc together comprise between about 0.5 and 70 weight percent of said composition.

9. The composition of Claim 8 wherein said catecholic butane comprises from about 0.1 to about 40 weight percent of said composition.

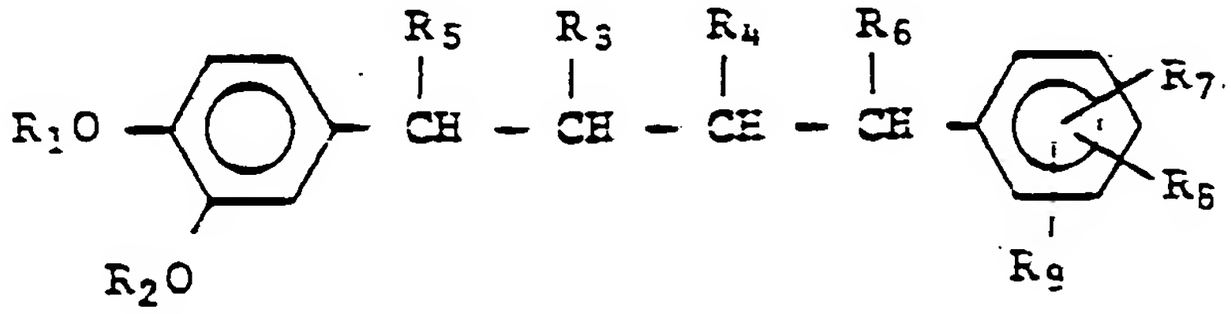
10. The composition of Claim 8 wherein said ionic zinc comprises about 0.1 to about 30 weight percent of said composition.

11. The composition of Claim 1 wherein said catecholic butane is 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane or 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane or mixtures thereof and said source of ionic zinc comprises zinc chloride.

12. The composition of Claim 11 comprising a pharmaceutically acceptable carrier and wherein said catecholic butane and said zinc chloride together comprise between about 0.5 and 80 weight percent of the composition.

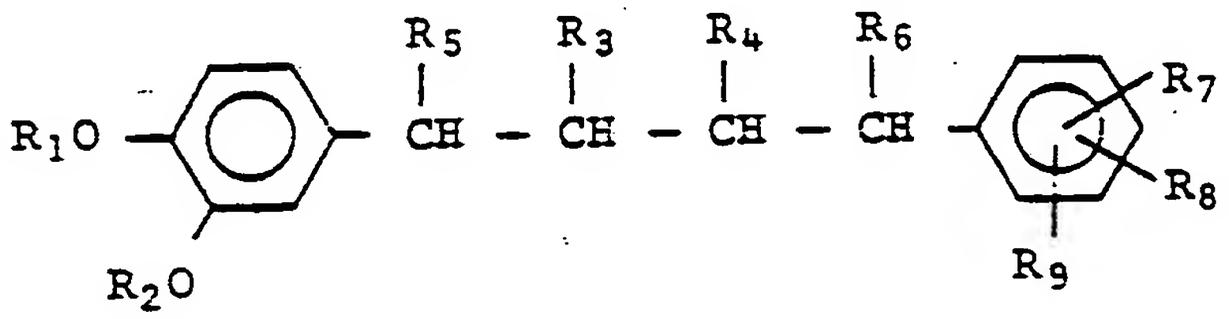
13. The composition of Claim 12 wherein said molar ratio is between about 3 to 1 and about 1 to 15.

14. A pharmaceutical composition suitable for applying to an afflicted situs comprising, in admixture with a pharmaceutically acceptable carrier, (a) a mixture of (i) a catecholic butane of the formula:



wherein R_1 and R_2 are independently H, C_1-C_6 alkyl, or a C_7 or lower acyl;
R_3 and R_4 are independently H, CH_3, or C_2H_5;
R_5 and R_6 are independently H or OH; and
R_7, R_8 and R_9 may be attached at any separate position C_2-C_6 of the benzene ring and are independently H, OH, or OR_1 wherein R_1 is as defined above;
and (ii) a non-escharotic concentration of a pharmaceutically acceptable source of ionic zinc;
(b) a zinc salt or chelate of said catecholic butane; or
(c) a mixture of (a) and (b) wherein in (a), (b) and (c) together comprise between about 0.5 and 80 weight percent of said composition and said catecholic butane and said ionic zinc each independently comprise between about 0.1 to about 40 weight percent of said composition with the proviso that the weight ratio of said catecholic butane to said ionic zinc is at least about 1 to 10.

15. A pharmaceutical composition adapted for applying to an afflicted situs comprising in admixture with a pharmaceutically acceptable carrier (a) a mixture of (i) a catecholic butane of the formula:



wherein R_1 and R_2 are independently H, C_1-C_6 alkyl, or a C_7 or lower acyl;
R_3 and R_4 are independently H, CH_3, or C_2H_5;
R_5 and R_6 are independently H or OH; and
R_7, R_8 and R_9 may be attached at any separate position C_2-C_6 of the benzene ring and are independently H, OH, or OR_1 wherein R_1 is as defined above;
and (ii) a non-escharotic concentration of a pharmaceutically acceptable source of ionic zinc, at least one of (i) and (ii) being present in a concentration effective to inhibit the proliferation of abnormal cells on or in tissue when the composition is applied to the tissue at the situs of said abnormal cells and the other of (i) and (ii) being present in a concentration effective to enhance the abnormal cell proliferation inhibiting activity of the former; or (b) a zinc salt or chelate of said catecholic butane in said abnormal cell proliferation inhibiting concentration; or (c) a mixture of (a) and (b).

16. The composition of Claim 15 comprising said mixture of said catecholic butane and said source of ionic zinc.

17. The composition of Claim 15 wherein said catecholic butane is selected from the group consisting of 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dimethoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dibutoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-diacetoxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane; 1,4-bis(3,4-dihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl)-4-(3,4,5-trihydroxyphenyl)butane; 1-(3,4-dihydroxyphenyl)-4-(3,4,5-trihydroxyphenyl)-2,3-dimethylbutane; and 1-(3,4-dihydroxyphenyl-4-(2,5-dihydroxyphenyl)butane.

18. The composition of Claim 15, wherein said catecholic butane and said ionic zinc are present in a molar ratio of between about 5 to 1 and about 1 to 20.

19. The composition of Claim 15, wherein said catecholic butane is 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane, 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane or mixtures thereof.

20. The composition of Claim 15, wherein said source of ionic zinc is zinc chloride, bromide, iodide, nitrate, phosphate, sulfate, acetate benzoate, citrate, caprylate, gluconate, or a mixture thereof.

21. The composition of Claim 20, wherein said source of ionic zinc is zinc chloride.

22. The composition of Claim 15, wherein the carrier is a semi-solid or liquid.

23. The composition of Claim 15, as a semi-solid or solid suitable for topical application.

24. The composition of Claim 15, wherein said catecholic butane is present therein at a concentration of from about 0.1 to about 30 weight percent of the total composition.

25. The composition of Claim 15, wherein said ionic zinc is present therein at a concentration of from about 0.1 to about 30 weight percent of the total composition.

26. The composition of Claim 15, wherein said catecholic butane is 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane or 1,4-bis(3,4-dipropionyloxyphenyl)-2,3-dimethylbutane and each of said catecholic butane and said zinc chloride are present therein at a concentration from about 0.1 to about 30 weight percent of the total composition and in a molar ratio of catecholic butane to zinc chloride of between about 3 to 1 and about 1 to 15.

27. The composition of Claim 26, as a semi-solid or solid.

28. A method for inhibiting the proliferation of abnormal cells in a mammal which comprises applying directly to the situs of the abnormal cells an amount of the composition of Claim 1 effective to inhibit said proliferation.

29. The method according to Claim 28, wherein the situs of the abnormal cells is the skin.

30. The method according to Claim 28, wherein said abnormal cells are a solid tumor.

31. The method according to Claim 30, wherein the tumor is in or on the skin.

32. The method according to Claim 29, wherein said composition is applied topically to the situs of the abnormal cells.

33. The method according to Claim 28, wherein said composition is injected into the situs of the abnormal cells.

34. The method of Claim 33, wherein said abnormal cells are a solid tumor and said composition is injected into said tumor.

35. The method of Claim 29, wherein said abnormal cells are a premalignant skin disorder.

36. The method of Claim 35, wherein said skin disorder is actinic keratosis.

37. A method of promoting the healing of a lesion in the tissue of a mammal which comprises applying to said lesion an amount of a composition according to Claim 1 effective to promote the healing thereof.

38. The method of Claim 37, wherein said lesion is a draining wound.

39. The method of Claim 38, wherein said composition promotes the granulation of tissue in the surface of the lesion.

40. The method of Claim 38 wherein said catecholic butane is 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane and said source of ionic zinc comprises zinc chloride and wherein said catecholic butane and ionic zinc together comprise from about 0.5 to about 80 weight percent of said composition.

41. The method of Claim 37, wherein said lesion results from acnes.

42. The method of Claim 37, wherein said lesion results from osteomyelitis.

43. A method for inhibiting the proliferation of a microorganism said method comprising applying to the situs of growth of said microorganism a proliferation inhibiting amount of the composition of Claim 1.

44. The method of Claim 43, wherein said microorganism is selected from the group consisting of Propionibacterium acnes and Staphylococcus aureus.

45. The method of Claim 44 wherein said catecholic butane comprises 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane and said source of ionic zinc comprises zinc chloride and wherein said catecholic butane and ionic zinc together comprise between about 0.5 and about 80 weight percent of said composition and the molar ratio of said catecholic butane to said ionic zinc is between about 5 to 1 and 1 to 10 with the proviso that said zinc chloride is present in less than an escharotic amount.

46. A method for enhancing the retention time of a catecholic butane in tissue at the situs of topical application said method comprising applying said catecholic butane as a composition according to Claim 1 which composition contains an amount of ionic zinc effective to enhance said retention time.

47. The method of Claim 46, wherein said source of ionic zinc is zinc chloride, bromide, iodide, nitrate, phosphate, sulfate, acetate, benzoate, citrate, caprylate, gluconate, or a mixture thereof.

48. The method of Claim 46 wherein said catecholic butane is 1,4-bis(3,4-dihydroxyphenyl)-2,3-dimethylbutane and said source of ionic zinc comprises zinc chloride.

49. A method for enhancing the oxidative stability of a catecholic butane in the composition of Claim 1 said method comprising providing a concentration of said ionic zinc effective to retard the formation of oxidative by-products from said catecholic butane.

50. The method of Claim 49, wherein said ionic zinc is present in an amount sufficient to decrease the decay rate of at least a portion of semiquinone free radicals formed from said catecholic butanes.

51. The method of Claim 49, wherein the molar ratio of said ionic zinc to said catecholic butane is greater than about 1 to 5.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/01740

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 33/30, 33/24, 33/34, 33/06, A61K 31/05,
U.S. CL: 424/145, 424/131, 424/140, 424/154; 514/736,514/731

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/145, 131, 140, 154; 514/736, 731

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US,A, 4,229,437 (LIKENS) 21 October 1980 See the entire document.	1-42 & 46-51
Y	US,A, 4,315,916 (LIKENS) 16 February 1982 See the entire document.	1-42 & 46-51
Y	US,A, 4,406,881 (LADANYI) 27 September 1983 See the entire document.	1-27 & 43-51
A	D. Burk, et al, Radiation Research Supplement Vol. 3, published 1963, pages 212 to 216. See the entire document.	1-51

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
20 October 1986	19 NOV 1986
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	J.W. Rollins

Form PCT/ISA/210 (second sheet) (May 1986)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	H. Willshaw, et al, British Journal of Opthalmology, Vol. 67, published 1983, pages 54 to 57. See the entire document.	1-36 & 46-51
Y	C. Smart, et al, Rocky Mount. Medical Journal, Published 1970, pages 39-43. See the entire document.	1-36 & 46-51
Y	O. Gisvold et al, Journal of Pharmaceutical Science, Published 1974, pages 1905 to 1907. See the entire document.	1-36 & 46-51
Y	M. Dyer "An Index of Tumor Chemotherapy" Published March 1949 (National Cancer Institute). See pages 10 to 12, 19, 40, 41, 79, 80 and 81	1-36 & 46-51

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . ... . , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-36 drawn to method of inhibiting proliferation of abnormal cells; Class 424 Subclass 145;

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone Practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.